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PRINCIPLES OF DEVELOPMENT

A Text in Experimental Embryology

By

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UNIVERSITY OF CHICAGO

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PREFACE

The ground covered by this book is still in the condition of new country in pioneering days, a state of flux with rapid and unpredictable advances and fascinating prospects. A guide book through this new field must take into account the needs of both the visitor and the prospective settler. The former wants concise information about the progress already made; the latter looks for advice as to how to proceed further in a profitable manner. It has been tried, therefore, in this book to combine a review of past achievements with a preview of presumable future trends by representing the facts on record as a cross-section through a living stream of problems evolving from the past into the future. Thus the problems have been put in the center of interest and the individual facts rated by the light they shed upon these problems and the contributions they have made, or promise to make, toward their solution. The growth of our science has been treated as the growth of an organism whose food is the ever increasing mass of factual knowledge. Just as food must be digested, resorbed and assimilated before it can become incorporated in the living body, so the recorded data of our experience supply merely the raw material for the growth of science; they must be worked in, not simply stuck on, to the existing body of knowledge, if they are to increase its substance rather than its dead weight. The conviction that in order to become science, facts must be collected *and* interpreted, has determined the attitude taken in this book as well as the selection and disposition of its subject matter.

No attempt was made to relate all the facts known in any department of the field exhaustively; comprehensiveness has been renounced in favor of a continuous and coherent presentation. It has been deemed preferable to take up fewer points more thoroughly than to deal with a greater number more summarily. Wherever one or two pertinent examples appeared to illustrate the case under consideration with sufficient clarity, the enumeration of further examples was omitted, even though a considerable stock of additional evidence may have been at hand. Accordingly, the space devoted to any given subject does not reflect the amount of attention and work accorded to it in the literature. Many fields rather extensively treated in the literature had to be condensed, while others, much more poorly explored, have been given enlarged consideration. This was done in order to maintain a certain measure of proportionality between the emphasis placed upon certain problems and their significance.

The selection of examples was influenced, aside from their pertinence, by a number of considerations. Whenever there were several examples of equal value and clarity to choose from, preference was given to those which fitted best into the context, or which were most prominent historically, or which illustrated the point most poignantly, or which were best documented, or which were, other things equal, closest to the author's own practical experience. It was partly for this last reason, when an organ was to be chosen whose developmental analysis could serve as model for the study of mechanisms of development in general, that the choice fell on the nervous system. Respecting the distinction between principles and mechanisms, which will be explained in the text, the discussion of this example of mechanisms has been put into a separate section (Part IV). It is regrettable that lack of space has prevented the inclusion of further examples of the many lines along which recent research has met with brilliant success; the most tangible omissions are: the mechanisms of maturation, sex differentiation, metamorphosis, pigmentation and color patterns, notably in the skin of vertebrates, feathers of birds, and wings of moths and butterflies.

In spite of unavoidable restriction, however, the subject matter extends considerably beyond the limits indicated in the subtitle of the book, which may be considered as a designation *pars pro toto*. Embryology covers only part of development, and the experimental method is not the sole tool of analysis. Hence, post-embryonic phenomena (functional adaptation, aging, regeneration) have been included among the topics, and analytical methods other than experimental have been given some attention. Furthermore, as consideration of the factors of development presupposes some familiarity with the facts of development, an outline of the latter (Part I) has been inserted, preceding the methodical (Part II) and analytical (Part III) sections.

Strict *topical* organization of the subject matter was abandoned in many places for *logical* organization. Considering that a single fact usually bears on a variety of problems, and that each single problem receives light from a wide array of facts, the recurrent use of the same fact in different connections seems justifiable. Moreover, the presentation of the same or similar data in different places under different angles will obviate the formation in the reader's mind of rigid associations between these data and irrelevant circumstances with which they may incidentally appear associated in the text.

The bibliography is as fragmentary as the selection of examples which it serves to document. Assuming that those taking a more than cursory interest in the field will want to consult the original literature directly, care was taken, wherever possible, to refer to monographs, reviews, or, at least, the most recent articles for a more detailed list of references. Again, as in the case of the examples, the bibliography does not express the true proportion in which different authors have contributed to the promotion of the field, and apologies are due to those who, for reasons of expediency, had to go unnamed.

I am greatly indebted to those of my colleagues, assistants and students who have helped in the preparation and revision of the manuscript. I am also most grateful to Drs. A. Dalcq, H. B. Fell, V. Hamburger, R. G. Harrison, E. B. Harvey, A. R. Moore, D. Price, G. Vandebroek and B. H. Willier for supplying me with some of their original photographs, drawings or preparations for the illustrations of the book. Acknowledgement is due to the publishing houses of Akademische Verlagsgesellschaft, Leipzig; Gebrüder Borntraeger, Berlin; Gustav Fischer, Jena; Methuen & Co., London; Julius Springer, Berlin; and Henry Holt & Co., New York; for the permission to reprint figures from their copyrighted publications.

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PART ONE

THE PHENOMENA OF DEVELOPMENT

A DEFINITION OF DEVELOPMENT

What is development? It seems trivial to ask this question. The term may seem too familiar to require further explanation. Does not everybody have some notion of what development implies? Undoubtedly, most of us have. But when it comes to formulating these notions, they usually turn out to be very vague. This renders them unfit for scientific use. While in everyday life, we may acquiesce in such statements as the one Lord Bryce once made with regard to political systems: "Though we cannot define either oligarchy or democracy, we can usually know either the one or the other when we see it," scientific formulations demand greater precision. Of course, we seem to know development "when we see it," but certainly all of us do not see it in the same light. Hence, when speaking of "development," everybody has probably something slightly different in mind, and we will end up in dissent. Let us, therefore, follow sound scientific maxim and *define* our subject before we analyze it. Ill-defined, vaguely defined, or undefined terms are a scientific curse; they make scientific language oracular instead of unequivocal, hazy instead of clear, and degrade all scientific work described in such language.

We shall try, therefore, to arrive at an agreement as to what should be considered as development, and what should not.

Repetitive, or physiological, events.

Our life can be measured by a double standard: on a short range and a long range scale. The former applies to our daily activities, the latter to the great lines of our lives. The short range events comprise those elementary functions of vital concern which make up our daily routine; in this category we count breathing, eating, digesting, moving, excreting, sleeping, as well as sensations, emotions, moods. We are aware of having performed these acts in essentially the same fashion, minor fluctuations notwithstanding, as long as we can remember. We know that they have their proper rhythms, recurring at the rate of seconds, minutes, hours, or days, as the case may be. In a good many instances the periodicity is quite strict, indicating a regular alternation of processes of opposite sign — the one leading away from, and the other leading back to, a basic standard condition. Inspiration vs. expiration in breathing; systole vs. diastole in the heart beat; contraction vs. relaxation of muscle; filling vs. discharge of the bladder — these are examples of such antagonistic changes. There are other cases in which both the positive and the negative phases go on simultaneously instead of in alternation, so that a stationary condition results that might easily be mistaken for stagnation, though it is all activity. Skin is continually worn off and shed in tiny scales at its outer surface, while it is restored at the same rate from the inner layers. In still other cases the changes are much less regular. Body weight, body temperature, irritability, stamina (tonus) and many other characters fluctuate in accordance with the accidental fluctuations of weather, nutrition, work, and the like. Yet in regard to all the phenomena mentioned, be they periodic or stationary or variable, we have the distinct impression that during the brief periods within which they occur and recur, *our basic condition remains unaltered and stable.*

Permanent, or developmental, changes.

This is no longer true when a long range view is taken. As soon as we try to cover several years of our life at one glance, all those short range happenings vanish at once from the picture, just as the details of a landscape fade as we gain distance, while at the same time the gross lines emerge. The gross line of our life, however, reveals *constant, though slow, change of our bodily and mental norm,* rather than the static condition impressing the short range reviewer. When we compare records (measurements or photographs) of various stages of our life taken at sufficiently great intervals, we note that some marked change has taken place in the course of time, and we recognize further something which is very characteristic of this change: first, its course is unidirectional, progressive, never reversing its trend and never returning us to an earlier stage; and second, its rate on the whole decreases with increasing age.

Development vs. physiological events.

Evidently it is these *slow* and *progressive* changes to which the term "development" refers, in contradistinction to the *fast* and *repetitive* changes attending functional, i.e., "physiological," activity. The first step toward a definition of development, therefore, seems to be to delimit its province against that of physiological function.[1] Three points come to our mind as possible criteria on which to base a distinction between *developmental* and *physiological* phenomena: (1) their different biological significance; (2) their different time scale, and (3) the progressive character of development versus the conservative character of physiological function. Let us scrutinize more closely the pertinence and merits of these distinctions.

Development preparatory to functioning.

We are aware of a sort of reciprocal relationship between developmental and physiological activities; the former prevail during earlier, particularly prenatal, stages of life, while during later, particularly postnatal, stages there seems to be more functioning and less developing. This does not mean that the life span is sharply divided by birth into a developmental and a functional phase; development continues into adult life and, conversely, many physiological functions of the adult can be traced far back into the embryonic stage.

[1] The term "physiological" is used in this book in the restricted sense of something pertaining to the *operation* of a functional organism; in other words, to the kind of activities with which Physiology proper deals.

However, though there is no abrupt change from developmental to physiological activities, the preponderance shifts gradually from the former to the latter as the individual grows older. There is more developing in the beginning and more functioning in the end, and this fact, that development, generally speaking, is *antecedent* to function, has been interpreted by biologists to mean that development *prepares* function. The biologist thereby introduces the element of *purpose* into the concept. He proposes to look at the organism as if it were an industrial establishment: first it is erected, then it is used. Development would correspond to the construction, and physiological function to the operation of the plant. In the life history of an eye, for instance, we can easily tell the processes preparing the structural and functional equipment required for vision from those which in the finished apparatus do the "seeing." The former set the stage, as it were, and the latter do the acting.

It will be noticed that in this classification processes are rated not by their inherent properties but according to whether they are of direct or indirect service to the organism in its final functional condition. If they are of *future* concern, they are regarded as *developmental;* if they are of *immediate* service to the operation and maintenance of the body, they pass for *physiological.* According to this thesis, developmental processes make sense only in that they build up something which will be of use in the future, while their immediate profit to the organism would be nil. The stress lies in the contrast between *prospective* and *actual* value.

The prospective value of development.

Take our muscles, for example. An impulse travels down a nerve and into a muscle, where it activates chemical and colloidal and electrical changes of such specific character that they make the muscle contract. The muscles are fastened to the skeleton so appropriately that contraction produces orderly movements, and locomotion results. Going back to some early developmental stage, we find a forerunner of the muscle though no nerve is as yet there to excite it, and if there were a nerve, the muscle could not as yet contract, and if it could contract, the joints would not as yet be in a condition to permit motion. However, the developmental processes elaborate muscle and nerve and skeleton and their mutual relationships in such — one might almost say, premeditated — fashion that each part, when called upon to act in later life, will be in a position to execute appropriately its prepared role as part of a functional organism. When the need for locomotion arises, the apparatus of locomotion must be ready; thus it must have been adequately provided for by previous planning and construction. All of the structural details of a wing feather, so marvellously adapted to the requirements of flight, have been perfected long before the bird has ever taken to the air. The intricate apparatus of mating in the snail is ready for use at the very first time two animals copulate. Likewise, the stinging apparatus of a bee is complete when it stings, and the spinning mechanism of the spider when it spins its first thread, and the digestive glands of the intestine when food is ingested for the first time.

This is all so obvious that it hardly needs mentioning — so obvious, indeed, that reference to the merely prospective value of developmental functions has become a standard practice. Yet we must look at this practice with a certain reserve for reasons of facts as well as of principle. As for facts, we know a number of obviously developmental processes which never lead to functional products. As a matter of principle, there is a general scientific aversion to so-called teleological formulations (imputing aims into natural processes).

Developmental features without functional significance.

In the first place, many instances can be cited in which development is decidedly not preparatory to functioning. There is growth, for example, taken in the restricted sense of increase in mass. In earlier stages of development it is undoubtedly functionally significant. The proper amount of material required for the formation of functional organs must be procured; the bones of the leg must have a certain size if they are to support the bulk of the body; the layer of fat in the skin of the seal must be sufficiently thick to act as heat insulation; the amount of blood produced must be adequate for the supply of the tissues and the filling of the vessels. However, once those indispensable limits are reached, a further continuation of growth seems of minor importance so far as functioning is concerned. Nevertheless, growth goes beyond the limit — for what use, we do not know. Each species contains dwarfs and giants, and apparently both function satisfactorily. Obviously, then, there are features of development which are functionally irrelevant.

Then, there is the case of the so-called *rudimentary* organs which are just there without exhibiting any recognizable physiological function; for instance, the immobile and limp extra toe on the leg of Houdan fowl and some shepherd dogs, or the human appendix. There is, furthermore, the case of *transitory* organs which after having been developed to a certain degree of perfection, disappear again without having ever assumed functional activity; for instance, the notochord of vertebrates. Certainly, in these instances, development could not be regarded as preparatory to physiological functioning.

Inadequacy of the teleological rating.

Our second objection is directed against the imputation of an aim in development as such. When we ascribe future significance to developmental events, and classify them according to their *aim*, we surmise that they have an aim. Viewed objectively, the organism is just one long list of activities; it remains silent, however, as to which of them are means to an end, and which are ends in themselves. The decision in each case is left to our judgment as observers; but this judgment is subjective, limited, and often enough fallacious.

For instance, what in one group of animals appears as the final functional stage of an organ, may in some related group merely be a passing event in the course of development devoid of actual functional significance. The mesonephros of amphibians represents the definitive kidney of the adult; its development could, therefore, be described as preparatory to the establishment of the excretory function. The same mesonephros, however, in the mammal not only fails to function as an excretory organ but proceeds to develop into a functionally different organ, namely, part of the male reproductive system. Now, in both forms the formative materials arise from the same source, undergo the same modifications, follow the same sequence of stages; in other words, the *processes* forming the mesonephros, to all practical purposes, are identical in amphibians and mammals. Is there any justification in concealing this factual identity by placing all emphasis on the difference in their presumable *role*? Does it change the character of the processes that their product — the mesonephros — is a functional physiological implement of a working organism in one case, and merely a temporary scaffolding upon which later developmental work proceeds in the others? Apparently not; certainly no more than the erection of a trestle appears to us in a different light depending on whether it is intended to function as a railroad bridge or merely as framework upon which to build a concrete dam.

We feel that if the *actual processes* are the essential phenomena, it would be quite beside the point to rate them according to what we surmise to be their purpose. We must realize that if we ascribe purpose to a process just because we happen to know its outcome — and this is precisely what a teleological explanation amounts to — we go beyond the plain facts and abandon the safe position of objective recorders for the unsafe one of subjective expounders of nature. Our prime efforts must be directed toward defining and explaining the phenomena of nature *from their objec-*

tively demonstrable properties rather than from those which as subjective observers we are inclined to ascribe to them. Development, too, must be defined on the basis of what is going on during the process, rather than of what we think to be its purpose.

The time scale of physiological and developmental events.

Let us, therefore, return to the other propositions and consider the two remaining criteria of development: time scale and directiveness. To consider the former point first — developmental activities have impressed us as of considerably slower progress than physiological activities. To illustrate the class of truly physiological processes, we choose a group of rhythmic phenomena whose rates can be conveniently estimated from the duration of a single period. In some common activities they are of the following order of magnitude:

TABLE 1

excitation of nerve	10^{-3}–10^{-2} sec.
beat of cilia	10^{-1} sec.
beat of heart	1 sec.
contraction of smooth muscle	10 sec.
secretion of glands	10^{2}–10^{4} sec.

According to these figures, the time within which the elements of the listed organs pass through one complete cycle of activity ranges from one thousandth of a second up to several hours, or if one wishes to include the daily fluctuations of our physiological condition, up to one day.

In contrast to these physiological phenomena, developmental events ordinarily proceed at a much slower rate. That is, in order to reveal perceptible changes their observation must be extended over considerably longer periods. The appropriate time unit by which to measure them is a day or a week or a month or a year. Furthermore, while the rate of physiological activities does not vary greatly, developmental events slow down very markedly with increasing age.

Intergradations.

The difference in time characteristics between physiological and developmental processes is sufficiently clear if we consider the paradigms of each type. If, however, a more comprehensive view is taken, one comes across a large group of borderline cases in which this difference fades. First, we know a number of developmental events proceeding with physiological rapidity. The embryo of the developing snail which has grown straight and symmetrically up to a certain stage becomes bent rather suddenly into the asymmetrical torsion characteristic of the adult; this whole change is completed within minutes. The very conspicuous changes taking place in an egg within a few minutes after its fertilization must also be remembered in this connection. On the other hand, changes of our physiological state can be so slow as to extend over weeks or months; as examples we mention the monthly periodicity of menstruation, the seasonal cycles in the behavior of many animals, and the reactions of our body in disease which often require weeks to become discernible and again weeks to disappear during recovery. Consequently, no crucial distinction between developmental and physiological processes can be based on the criterion of time rates.

Phenomena in the service of both development and function.

This reduces our choice to the third possibility suggested, namely, that of telling developmental from physiological events by the *progressive* character of the former, as against the *stationary* character of the latter. That such a distinction is feasible, is best illustrated by cases in which the same vital activities are engaged in both categories.

Secretion is a case in point. Secretion of saliva by glands of the oral cavity is a typical physiological act. On the other hand, the secretion by the cells of the embryonic retina which fills the interior of the eye and produces the vitreous body is clearly development. Naturally, so far as the secreting cells are concerned, there is no essential difference between salivation and the production of the vitreous humor. And yet one feels quite justified in classifying the former as physiological and the latter as developmental. Why? Obviously because salivation has only passing effects of no permanent consequence while the secretion of the vitreous body results in a lasting addition to the equipment of the organism.

In the calciferous glands of the earthworm, calcium salts are stored and periodically released for some physiological use, possibly connected with metabolism. In contrast to this stationary calcium reservoir we find that the calcium deposits in the shells of molluscs increase more or less steadily. Accordingly, we class the former as of physiological, the latter as of developmental character.

Cells often accumulate in a localized region of the body in response to some local stimulus; white blood cells, for instance, gather around foci of injured tissue, but after having done their work there, they disperse again, and we may consider the whole affair a closed physiological incident. Why physiological? Because once it is over, the body has returned to its previous condition. Under certain circumstances, however, aggregated cells fail to scatter and a permanent change has been caused to the organism. Consequently we call the reaction developmental; indeed, the formation of a new organ or a tumor can be the result.

A macrophage (phagocytic blood cell) often sends out a pseudopodium to ingest some foreign particles, as do also the intestinal cells of certain lowly animals in order to seize food fragments; since the protruded process is withdrawn afterwards and the cell returns to its original shape, we call this change physiological. On the other hand, the elongate pseudopodium-like process which is protruded from an embryonic nerve cell and gives rise to the axis cylinder of the nerve fiber, tends to persist; hence, this phenomenon must be designated as developmental.

In lower vertebrates the pigment surrounding the visual elements of the retina migrates in response to illumination, spreading out in bright light and contracting in the dark; the amount of pigment present is not altered in the reaction, and the effect is plainly physiological. In contrast to this, consider the lasting effect of light on animal coloration. In many animals the presence of light is prerequisite for the formation of dark-colored granules in the skin; wherever this is the case, illumination does not simply control the distribution of existing pigments, as in the preceding example, but stimulates the production of new colored matter which accumulates and leads to progressive darkening of the skin. The cumulative effect marks the phenomenon as developmental.

Development is progressive — physiological function conservative.

We learn from this heterogeneous collection of examples that the same kind of activity (e.g., secretion, cell aggregation, cell transformation) can appear at one time as a physiological and at another time as a developmental phenomenon. The persistence of the effects serves as the criterion. *If an effect passes without leaving a trace, it is physiological; if it leaves a permanent residue in the organism, it is developmental.* Changes of the former class are fleeting; each progressive step is cancelled by a simultaneous or subsequent regressive step of equal momentum, and the basic condition of the organism is left unaltered. This is illustrated by such antagonistic processes as oxydation and reduction; swelling and shrinking; contraction and relaxation (muscle); polarization and depolarization (membrane); aggregation and dispersion (cell). In contrast to these, the developmental changes are cumulative and progressive; they produce new structures, new shapes, new configurations, new proportions, new relations, rather than periodically repeating old ones as is typical of physiological processes.

This classification seems on the whole more satisfactory than the ones previously suggested. Although it still takes into account the *outcome* of the processes which are to be defined rather than their nature, one cannot fail to recognize that this recourse to outcome is altogether on a different level of scientific respectability than are the anticipatory references contained in the teleological definitions of development outlined before. For the actual outcome of a process can be recorded, measured and described in perfectly objective terms without the questions of biological significance, meaning and aim entering at all.

Evidently there is no definition of development that could completely renounce all reference to the final result. After exhausting all possibilities of fixing the limits between development and other vital processes, we find it impossible to identify developmental processes solely on the strength of what is going on, without taking into account the net result. This ought to convince us that *no vital processes are in themselves either specifically developmental or specifically physiological.* Consequently, a fundamental difference between development and function, in the sense of becoming and being, cannot be admitted, notwithstanding the practical value attached to the distinction. *Developmental and functional processes are essentially of the same making.*

For practical purposes, however, we will retain in this book the criterion of *progressiveness,* as it was explained above, to signify development. Gained by abstraction as the common denominator of a wide array of typical developmental phenomena, this criterion has proved more pertinent than others; in order to be consistent, we shall apply it even to those borderline cases which seem refractory to any sort of definition. Sometimes an almost imperceptible change will be sufficient to bring a process from the range of our physiological into that of our developmental definition, and vice versa.

Borderline cases.

A few examples may illustrate this. The formation of blood in the embryo and growing organism is, according to our definition, and in any definition, a developmental process; the number of blood cells is constantly increasing, and they undergo changes of unmistakably progressive character. But even at a time when the production of new cells is still very intense, one can already note that old cells begin to be destroyed. So long as production exceeds destruction, the actual gain to the organism continues, but as soon as a state of equilibrium is reached in which production and destruction balance each other, the volume of the blood has become stationary, and all further activity ought to be rated as physiological. This conforms to the usage according to which, for instance, secretory activity in so-called holocrine gland cells is designated as physiological; these cells perish in the act of secretion but are continually renewed at the rate at which they are lost. One can see that in cases like these the distinction between developmental and physiological matters becomes rather artificial.

Finally, when it comes to appraising phenomena in which wear represents the regressive factor checking production, our judgment seems to run directly counter to the definitions to which we have committed ourselves. The incisors of rodents grow continuously, but they are also worn off continuously at their tips in the exercise of gnawing; since reproduction from the base is as fast as the distal reduction, they maintain a constant shape and size. Occasionally, however, an animal is observed in which the teeth are so improperly set that the upper and lower incisors fail to meet. Attrition thus being eliminated, growth continues unchecked producing weird spiral teeth of ever increasing dimensions. Now, if we cling to our definitions, we must say that an accident decides whether the process is of physiological or developmental order. Logically this is incontrovertible, but it can be rightfully questioned whether more than academic value attaches to this straining of definitions.

What is development?

To summarize, development is a complex of vital phenomena possessing certain peculiarities but by no means sharply outlined against the rest of vital activities. Accordingly, an unfailing definition of what is development, and what is not, has not been forthcoming. The best criterion available is *progressiveness*. Even this, however, loses its sharpness in the borderline field; if, nevertheless, it is applied in the following discussion as rigorously as if it were of absolute validity, it should be borne in mind that this is merely a matter of convention.

AN INVENTORY OF DEVELOPMENT

General Changes

The complexity of development.

Having established the confines of our subject, we shall now take stock of its contents. Development is a highly complex and truly synthetic affair. The observer to whom development of an organism appears like the sculpturing of a statue, as a single homogeneous event, is deceived by the smooth and harmonious manner in which the numerous and diverse component processes are amalgamated. But in reality the making of an organism is the work of a host of different elementary processes. Ample evidence to bear out this statement will be supplied in later parts of the book; for the moment, let us simply take the composite nature of development for granted. It warrants our asking for the constituents, and we shall now try to single them out, one by one.

GROWTH

Definitions.

The most striking difference between an adult and a child is that of size; the factor that has brought about this difference has been called *growth*. Growth means a developmental increase in the total mass of the body. We say *developmental* increase, because physiological fluctuations of body volume are not included in the term. When we gain weight without changing in length, we do not say we have grown. For, inasmuch as we may lose weight and return to our previous condition, the change must be considered as of a different order than growth in length from which there is no return. Consequently, fluctuations in the amount of water or of reserve materials (fat) stored in the body, although affecting weight, are not growth. Growth means *permanent* enlargement.

The rate of the increase is called *growth rate;* it equals the absolute amount of increase taken over a certain period of time, divided by the length of that period. This is called the absolute growth rate (V_a).

$$V_a = \frac{\Delta M}{\Delta t} = \frac{M_2 - M_1}{t_2 - t_1}$$

where M_1 is the mass of the body at the time t_1, and M_2 is the mass at a later time t_2. If growth is constant over the whole considered period, then V_a expresses the *true* growth rate; otherwise it merely represents the *mean* growth rate which approximates true growth rate the more, the briefer the interval, $t_2 - t_1$, is.

For use in comparative studies the absolute values of the increments are less instructive than are the relative or percentage values, in which we express the increase of mass in relation to the total mass already present. A gain of one pound marks a real achievement in a new-born baby, but goes unnoticed in a boy of sixteen. The absolute increase is the same in both but, calculated for the original masses, it will be somewhere around 15% in the former and 1% in the latter, and consequently of quite different import. The relative increment is defined as change in mass (ΔM) over initial mass (M):

$$\frac{\Delta M}{M} = \frac{M_2 - M_1}{M_1}.$$

A similar distinction as that between absolute and relative increment can be made between absolute and relative growth rates. Relative growth rate (V_r) is relative increment per unit of time, or, expressed differently, absolute growth rate divided by initial mass.

$$V_r = \frac{\Delta M}{M \cdot \Delta t} = \frac{V_a}{M}.$$

Absolute and relative growth rates.

It is self-evident that comparisons between different individuals or different forms of animals, or even between different stages of the same individual, must be based on relative rather than absolute growth rates. In other words, a giant and a dwarf cannot be said to grow at comparable speeds when both add the same number of pounds in the same period, but they can be considered as growing at corresponding rates if both increase their mass in the same interval by the same percentage. In the following table [1] a number of animals is listed with the time it takes each to grow to twice its original mass at birth or hatching; i.e., the time required for a 100% increase. This time is, of course, proportional to the mean relative growth rate.

TABLE 2

Fly larvae (Caliphora, 20° C.)	13 hours
Silk worm	68 hours
Rabbit	6 days
Pig	6–7 days
Sheep	10 days
Guinea pig	18 days
Horse	60 days
Man	180 days

According to these figures, a new-born rabbit of 60 grams and a new-born pig of 1500 grams grow equally fast, while a sheep grows eighteen times faster than a human individual of about the same initial weight (4000 g.).

Different forms of growth.

Growth goes on either steadily, as in vertebrates, or in spurts, as in many arthropods in which the limited space

[1] Reproduced from *Pütter*, 1911.

within the rigid integument of chitin prevents an effective expansion of the body except during moulting. Even in steadily growing animals, however, the growth rates do not usually remain constant; in addition to a general *decline,* they are subjected to more or less marked *fluctuations.* These can be irregular, but more often are periodical with a pronounced daily or seasonal rhythm. The regular alternation between periods of faster and of slower growth is expressed in the corrugated surface of the shells of snails and of the scales of fishes. Just as the pattern of tree rings reveals the life history of a tree, so the pattern of shells and scales furnishes us with an automatic record of the whole growth history of the particular individual, and the rhythmic alternation of thicker and thinner or of wider and narrower portions in the pattern indicates directly the seasonal growth cycles of successive years.

In some forms (e.g., fishes) growth continues during the entire life span; in others (e.g., mammals) it is arrested when the "grown-up" stage has been attained.

Degrowth.

There is also such a thing as an eventual decrease in mass subsequent to a prolonged period of growth, and this has suitably been called "degrowth." The term does not refer to that sort of shrinkage which occurs during the transformation of a tadpole into a frog through loss of water from the tissues which become denser without losing in dry weight, like a withering flower. Degrowth means an actual reduction of body substance other than water, and a reduction which extends beyond the range of ordinary physiological fluctuations. Some of it seems to occur in all forms with limited growth as a result of the cumulative destruction by wear of parts of the body which in the later stages of life is no longer compensated by adequate repair and regeneration. This by-product of ageing which appears as degeneration and atrophy is observed in many of even the highest animals although in them it remains always within very moderate limits. Degrowth of much more impressive extent occurs in lower animals (e.g., hydroids, flatworms, nemerteans, tunicates) whose bodies can undergo reductions to a fraction of their former size when they come under substandard living conditions (inanition, poor oxygen supply, etc.).

The constitutional factor.

1. *Every species has its peculiar growth parameters;* that is, rates and limits of growth, while varying greatly among different forms of animals, are nearly the same for the individuals of any one species. In other words, members of the same species, raised under identical conditions, grow with approximately the same speed, pass through the same size levels at approximately the same intervals, and reach approximately the same size limits. Although there is a certain amount of variation even within a single species, caused by slight initial inequalities among individuals as well as by the impossibility of insuring absolute identity of the conditions under which the different individuals are reared, the average nevertheless remains fairly constant, and the amounts by which the individuals deviate from the average remain also within definite and comparatively narrow bounds. (See the discussion of factors of size in Part III.)

The variation among individuals may be compared to the spread of buckshot: the initial direction of the shot is determined by the orientation of the gun barrel, and the general character of the resulting ballistic curve depends upon the intensity of the discharge, the weight and shape of the bullets, and a few more properties of the firing system. Although all the bullets contained in a single charge appear to be under identical conditions, their trajectories are more or less divergent and scatter around an ideal mean course. One cannot point at any single bullet and predict precisely which course it is going to take, but one can predict, for a given rifle, the *average* course that will be taken by the whole charge, and one can even determine the maximum amount by which an individual bullet is likely to deviate from this mean. Just as the course of the mean trajectories and the amount of scattering, other conditions being equal, are determined by the intrinsic properties of the firing system — its constitution, as it were —, so the peculiar characters of growth and the range of inter-individual variation are *constitutional* characteristics of each species.

The environmental factors.

2. However, just as the course and spread of the trajectories depend not solely on the constants of the firing system, but also upon external conditions, such as temperature and wind, so *growth is likewise the resultant of an interaction between the constitutional (intrinsic) factors of the growing system and the environmental (external) conditions under which it proceeds.* There are certain rules applying rather generally to the effects of external factors on growth.

a. Growth rates rise or decline as the temperature of the medium is increased or decreased; the medium may be air, water or the tissues and fluids of a maternal body. An example is furnished in the following table,[1] in which the time required for frog tadpoles to attain a given length is listed for various temperatures between 11° and 18° C. It can be seen that this time increases markedly as the temperatures drop; the longer it takes to reach a fixed growth mark, the lower has the growth rate been. Hence, the lower the temperature, the lower the growth rate.

TABLE 3

Length of Larvae 7.0 mm.		Length of Larvae 7.8 mm.	
Temperature	Hours	Temperature	Hours
18.1	99	18.1	109
15.4	128.5		
12.9	174.5	12.95	193.5
11.0	227.5	10.95	263.5

b. Growth rates vary also in accordance with the amount and suitability of available nutrition. The extent of this variation is less during stages in which the organism is subsiding on stored reserves (yolk of the egg, fat of the adult) than during periods in which food must be secured from an environment not overabundant in it. An example is shown in the following graph (Fig. 1).[1]

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[1] *Krogh,* 1914.

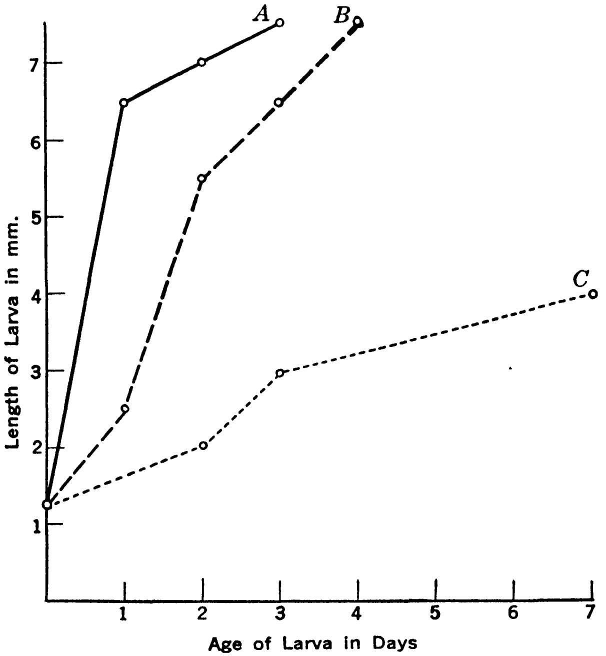

FIG. 1. Growth of fruit-fly larvae (*Drosophila*) in different nutrient media. (From *Baumberger*, 1919.)

A, Growth on food containing 24% yeast.

B, Maximum growth observed in medium containing 3–6% yeast.

C, Growth on hot unfermented aqueous banana suspension.

c. Growth rates, finally, depend upon a number of other stimulating or depressing factors, which arise from the interaction of organisms with one another. These may be called "social" factors in the broadest sense. They appear when numbers of individuals coexist in a limited space so that each individual becomes a part of the others' environment. The mutual influence on growth can be indirect, via the medium, or direct. Vigorous activity of a group can have a beneficial effect by raising the temperature of the medium; it can also act adversely by keeping the medium as well as individuals in continual agitation (Fig. 2). Competition for limited amounts of food has a depressing effect. Chemical emanations given off by the individuals and accumulating in the medium can be either advantageous or detrimental to growth, depending on their nature and concentration.[1]

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[1] *Baumberger*, 1919.

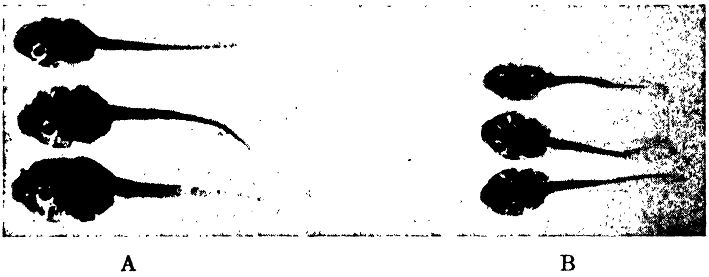

FIG. 2. Effect of agitation on growth. (From *Goetsch*, 1928.) Two groups of 15 frog tadpoles (*Rana esculenta*), 9 mm. in length, were reared in equal volumes of water (100 cc., steadily renewed at a rate of flow of 12 L. per hour). The medium was left undisturbed in one group (A), but constantly stirred in the other (B). The photograph shows the largest, smallest and one intermediate specimen from each group at the end of 4 weeks. The growth-retarding effect of the disturbance can be clearly recognized. × 2.5.

Intrinsic and extrinsic conditions of growth.

The two sets of growth controlling conditions outlined under points 1 and 2 are of essentially different order. The former set is *intrinsic to the growing system itself* and can conveniently be designated as "*constitutional*"; it accounts for the *specificity* of the growth rate of a given form, that is, for the fact that a form A will grow at a mean rate α, and a form B at a mean rate β under otherwise identical conditions. The other set of factors is *extrinsic to the growing system* and may be termed "*circumstantial*"; it consists of those external conditions prevailing during growth whose variation makes individuals of identical constitution grow at different rates. It will help to clarify the relationship between the constitutional and circumstantial elements in growth if one visualizes the former as "*growth potentials*" — because they establish a definite *predisposition* for growth — and the latter as "*growth circumstantials*," i.e., factors indispensable for the *realization*, but not responsible for the characteristics, of growth.

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[1] A competent discussion of these points is found in *Allee*, 1938.

A diagrammatic representation of growth.

We shall try to illustrate the situation in a stereogram in which we plot the constitutional growth tendencies, or "growth potentials," of different species on a vertical axis *P* and the circumstantial variation within each species on horizontal planes (*T*, *F*, Fig. 3). If we compare a number of different species growing under identical conditions, those with the fastest growth reveal the greatest intrinsic drive, i.e., growth potential, and are marked highest on the vertical axis; those with the slowest growth are marked at the lowest level of the axis and those intermediate are graded in between. This vertical gradation is merely to express the relative superiority or inferiority of the growing ability of a given species with regard to others under the same circumstances. But we know that the actual growth performance of an individual of given constitutional drive (growth potential) varies with the condition of the environment (growth circumstantials), particularly with temperature and food supply. This latitude is symbolized in the diagram by the horizontal expanse of the levels of growth potentials. If we plot in these planes temperature along the abscissa *T* and degree of nutritive adequacy along the ordinate *F*, each conjugated pair of values *T*, *F*, defines an actual growth rate at the given growth level *P*. For the sake of illustration let us agree to indicate the actual growth rate by a line connecting the origin of the *P* axis with the point corresponding to the prevailing *T* and *F* values; no precise numerical meaning should be attached to this representation except that the longer the line, the higher the rate of growth. We shall now briefly examine the implications of this model and select for discussion two species with growth potentials *A* and *B*, respectively, and $B > A$.

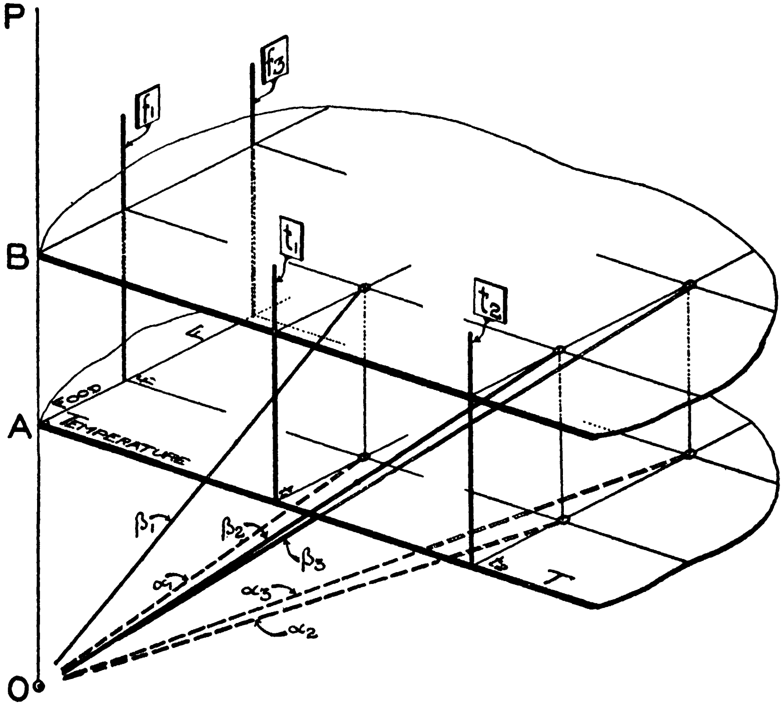

FIG. 3. Stereogram illustrating the relation between growth potentials and growth circumstantials. For explanation, see text.

1. The growth rate of a member of species *A* reared in a temperature t_1 and with a food supply f_1 (other conditions disregarded) is α_1; the growth rate of a member of *B* growing under the same conditions is β_1. It is evident that $\beta_1 > \alpha_1$.

2. The growth rate of a member of species *A* reared in a higher temperature, t_2, other conditions as in (1), is α_2; the growth rate of the same individual with the addition of improved nutrient supply (f_3) is α_3. Obviously $\alpha_3 > \alpha_2 > \alpha_1$.

3. A member of species *B* under the conditions listed in (2) would grow at rates β_2 and β_3 respectively, with $\beta_3 > \beta_2 > \beta_1$. Of course, compared with the *A* individuals under identical conditions, we would find $\beta_3 > \alpha_3$, $\beta_2 > \alpha_2$, just as we had (1) $\beta_1 > \alpha_1$.

4. It can be seen from the diagram that an individual of low growth potential *A* can still turn out of faster growth and larger size than one belonging to the intrinsically swifter species *B* if the former is attended by distinctly more favorable growth circumstantials.

The growth diagram should prove useful as illustration of the way in which one should conceive the relationship between growth potentials and circumstantials, particularly their decidedly non-additive character.

Growth limits.

Theoretically, the extent of each horizontal plane of circumstantials is unlimited since temperature and amount of food supply can be increased indefinitely. Practically, however, the range within which the growth of an organism can vary in accordance with the variation of the environment is narrowly circumscribed; it falls somewhere within the definitely restricted range of *tolerable living conditions.*

As for the temperature range within which growth can proceed, the tolerable extremes vary considerably among different species. Each species has its critical minimum below which, and its critical maximum above which no growth occurs; the lower limit is generally less sharply defined than the upper one.[1] There are similar limits regarding the nutritive factors; a definite minimum amount of appropriate nutriment is required in order that the organism may grow; this minimum lies somewhere above the level of fatal starvation. By making food available in amounts exceeding this indispensable minimum, growth may be accelerated, although the degree to which this is possible varies and is rather slight in some forms. In no form, however, can growth be driven, even by overabundant food supply, beyond a certain optimum rate of progress determined by constitutional factors of the growing system itself. This upper limit, above which growth cannot be enhanced may be defined as the "growth capacity" of the system.

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[1] For further details, see *Hesse*, 1937.

The growth norm.

The range enclosed by the tolerable extremes indicates the extent within which conditions are adequate for growth; the actual growth rate of an individual may come to lie anywhere within these confines. This does not mean, however, that *in nature* growth rates would be found in even distribution over the whole area. On the contrary, if one examines a sufficiently large number of individual cases, one discovers that the majority are crowded in the central portion of the tolerable range with increasing sparseness toward the borders. This uneven distribution is due to the fact that the environmental conditions under which a given form has proved fit to live and to grow do not ordinarily fluctuate so erratically as to reach the tolerable extremes anywhere as often as the optimum.

The central range containing the majority of cases corresponds to what we usually call the "norm," and the external conditions prevailing in it make up the "normal" environment. The "normal" growth range fades gradually into the marginal district of "abnormal" growth, either "supernormal" or "subnormal," which lies between the central district of highest incidence — the ideal norm — and the extreme limits of adequacy. The conventional distinction between "normal" and "abnormal" is based entirely on the selection of some arbitrary line as a marker — a practice expedient for the purpose of classification but without natural counterpart. Referring to our comparison between the variation of growth and the spread of shot, we can say that when we rate growth rates as either "normal" or "abnormal," depending on whether they lie on this or that side of a conventionally fixed mark, we act exactly as we do when we count or discount hits depending on whether they fall inside or outside of the bull's-eye of the target. What we have stated here for growth rates is equally valid for growth limits and, as a matter of fact, for all development. Everywhere the terms "normal" and "abnormal" indicate merely the relatively greater closeness or remoteness of an individual case in relation to an ideal mean used as a standard of reference. If, for instance, the climate changes and the average temperature range mounts, the growth norm shifts correspondingly and comes to include cases which previously would have been rated abnormal.

The relation between constitution and environment in development.

The diagram is instructive in one more way: originally pertaining to growth, it happens to be equally representative of the relative roles of constitution and environment in development in general. Constitution endows a system with a definite potentiality in the sense of a capacity to react, and environment furnishes the circumstances under which this potentiality can be realized and the reaction can occur. Systems of different constitution in identical environments develop differently; systems of identical constitution in different environments develop differently; only systems of identical constitution in identical environments give rise to identical developmental results. The diagram if correctly understood should prevent one from viewing constitution and environment as if they shared in development as equivalent and additive partners: such and such amount of the product to be credited to constitution (or, as it is often stated more vaguely, to intrinsic factors) and such and such amount to environment (or external factors). This would be just as inconsequent as the claim that the height of a fountain is the sum of two jets, one depending on the width of the nozzle and the other on the pressure of the water. If there is no nozzle, there is no fountain at all, whatever the pressure; and again, if there is no pressure, there is no jet in spite of an open nozzle. Similarly, constitutional factors predisposing for growth (and, more broadly speaking, for development), and environmental conditions making the growth (more broadly: developmental) potentials come true in a definite expression, are both prerequisite for every act of growth (or development). Without heat, there is no growth, and without food, no growth; but unless there is an intrinsic growth potential, there will be no growth, either. To sum up: *the environment controls the actual manifestation of intrinsic constitutional properties, without producing any such properties.*

Differential growth.

The manner in which we have dealt with growth in these pages is one of crude approximation. We have spoken simply of the total increase in mass. It is an obvious fact, however, that the developing organism does not add equal amounts of substance to each of its parts; otherwise a spherical egg with all its increase in mass would always remain spherical. Actually, growth proceeds at distinctly different rates in different portions of the organism, and hence, bulk determinations of the mass of the whole body do not give us a complete and true picture of growth. In other words, the *differential* character of growth is still to be accounted for in our picture. We shall return to this point shortly.

PHYSICO-CHEMICAL CHANGES

Before passing on to the differential side of development, another group of *general* changes must be considered which, like growth, involve practically every element of the body. Although less conspicuous than growth, they can be demonstrated by proper methods, and some of them even become rather obvious through their effects on the function and morphology of the organism.

Ageing.

Again, a comparison between a young and an old individual will best serve to point out the type of changes in question. First, in regard to function: as an individual grows older, his plasticity and adaptability diminish; his reactions become more stereotyped, and the limits within which he can adjust to new environment or to altered internal conditions become narrower and narrower; also the speed, if not the vigor, of many reactions declines. There is a gradual decrease in the capacity of the body to regain its normal equilibrium after injurious accidents, especially where regenerative power is required for the recovery. Everybody knows how much more slowly the healing of a skin wound or of fractured bones occurs in an old individual than in a young one. All this is commonly referred to as "ageing."

Now, there is not the least doubt that all these functional manifestations have a very material substratum; in other words, that they merely express underlying changes of the physical and chemical organization and constitution of the body. The decline of wound healing capacity of skin and bone is a very suggestive example.[1] The wrinkling and withering of skin with increasing age is a conspicuous index of a relative decrease in the ratio between water and the fibrous constituents of the tissue. A similar change, although more abrupt, occurs during the metamorphosis of amphibians, and there, too, the change is attended by loss, or at least deterioration, of regenerative capacity. In bone, likewise, the proportion between living plastic material and dead mineral deposits decreases more and more with increasing age; this explains why older bone is more brittle and also in part why the healing of fractures is more difficult. And if we study other tissues, we find a similar picture. It all builds up into a fairly consistent story, namely, that there is a gradual consolidation of the tissues, that is, a *condensation of their solid constituents accompanied by a loss of bound water*. These gross macroscopic changes, however, merely reflect corresponding changes to which the microscopic and sub-microscopic elements of the body are subjected.

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[1] For a thorough discussion of the problem of ageing, see *Minot*, 1908; *Mühlmann*, 1927.

The colloidal state of living systems.

Most of these elements, whether constituents of the living protoplasmic cell bodies or of the intercellular substances, exist in a physical state which we call *colloidal.* Since we shall have to refer to this fundamental property on many occasions, it is necessary to digress here to outline at least the principal features of colloidal systems.[1]

1. A colloid is a heterogeneous system; that is, it is made up of at least two distinct and immiscible types of substances, of which one is dispersed in the form of discrete particles within the other which forms a continuous matrix. If the particles of the discontinuous phase are of macroscopic or microscopic size, one does not call such a system a colloid, but speaks of suspensions and emulsions, depending upon whether the particles are solid or liquid. Muddy water, for instance, is a suspension of finely dispersed clay; milk is an emulsion of finely dispersed fat droplets. Only beyond the range of microscopic visibility does the realm of colloidal dimensions begin. As a matter of convention, we consider it to include all disperse systems in which the elements of the discontinuous phase measure between 1 and 100 $\mathrm{m}\mu$ ($1\ \mathrm{m}\mu = 0.001\mu = 0.000001\mathrm{mm}$). Since the resolving power of the ordinary microscope does not permit a discrimination of particles of such small dimensions, colloids appear homogeneous under the microscope. The continuous phase of the colloid, which corresponds to the solvent of an ordinary molecular solution and frequently consists of water or oil, is called the *dispersoid.* The discontinuous matter suspended in it is called the *dispersed phase,* and its individual elements are called *ultramicrons.* The degree of dispersion may be termed

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[1] For further information about the matter, see *Gortner,* 1938. A brief appraisal of the biological significance of colloidal structure will be found in *Lloyd,* 1932; *Seifriz,* 1938.

dispersity. For a given mass of dispersed material dispersity is obviously in inverse proportion to the size of the individual elements. For the inner surface separating an ultramicron from the ambient dispersoid the term *interface* is in use.

The inner surface of colloids.

2. Thus, the term colloid does not refer to a class of substances but rather to a peculiar physical state in which substances can appear under certain conditions. A system in the colloidal state has characteristic properties which distinguish it from both the macroscopic and the molecular order. The main distinction is the predominance of *surface* phenomena, i.e., phenomena produced by forces acting along phase boundaries; e.g., surface tensions such as prevail in the phase boundaries between water and air or between oil and water, etc. The reason for the prominence of surface activities in colloidal systems is that these systems possess an enormous total surface in a small volume of substance. This is simply a result of the high degree of dispersion. For, when a solid block of substance is broken up into a large number of fragments, every new division creates new surface by bringing to light parts formerly enclosed in the interior. For example, a cube with sides measuring 1 cm. in length has a surface of 6 sq. cm. When this cube is cut up into microscopic fragments of 1 cu. μ each, the total surface of these adds up to 60,000 sq. cm., which is more than the area covered by a 6 × 9 foot rug. If the fragmentation is continued until the particles measure only 1 mμ (which is the lower limit of colloidal dimensions) the total surface becomes a thousand times as great, i.e., 60,000,000 sq. cm. which is an area about half again as large as an acre. While the total surface is thus increased to ten million times the original value, the volume remains unchanged. This readily explains why surface forces gain so rapidly in importance with increasing dispersity, until in the range of colloidal dimensions they obscure all other forces.

One of the chief surface effects is *adsorption;* this is the capacity of the dispersed particles to retain in their surfaces substances contained in the ambient medium. Even a certain amount of the dispersoid itself becomes thus fixed to the dispersed elements. So, ultramicrons with affinity for watery dispersoids (so-called *hydrophil* colloids) are each invested with a shell of water which they carry with them as they move. Other substances have greater affinities for other solvents than for water and are, appropriately, called *hydrophobe.*

Coagulation.

3. Colloidal systems appear in two states, called *sol* and *gel,* comparable to the liquid and the crystalline states of molecular dispersions. In the sol state the colloid flows freely, that is, its ultramicrons can move about as freely as can the particles of an ordinary suspension. The ease with which they can be shifted with regard to one another is in inverse relation to the inner friction, which varies with the concentration and size of the elements and a number of other factors. Inner friction finds its expression in the *viscosity* of the system (see p. 126). Sols can change into gels, that is, they become solidified and assume a jelly-like consistency; the ultramicrons lose their free mobility by becoming joined to one another. The sol-gel transformation is known as *gelation* or *coagulation.* It can be brought about by physical means (cooling, change of electrical charge) or by chemical action (salts, enzymes). In some instances it is reversible, in others it is not. A gel can be represented as a spongy framework of ultramicrons joined together, with large amounts of the liquid dispersoid enclosed in the meshes. This constitution of a gel accounts for its remarkable elasticity.

Syneresis.

4. A dispersed system has a natural tendency to decrease its dispersity. This means that the particles of the discontinuous phase tend to aggregate into larger units. Figuratively speaking, the "grain" of the system becomes coarser. This trend marks a transition toward greater sta-

bility. If the colloid is left to its own devices, the transformation occurs at a steady but usually fairly slow pace. Coagulating agents speed it up, other agents slow it down, and can even completely arrest it. So-called "protective" colloids act in the latter capacity; they are substances which are adsorbed to the surfaces of the ultramicrons and thereby form protective coats through which the particles so enveloped are prevented from coming into contact and fusing with one another. In unprotected colloids, however, the dispersity decreases gradually; physical and chemical properties change concomitantly. Owing to this tendency even a gel lacks stability; after coagulation the ultramicrons continue to join into larger units so that a progressive separation of the colloid into its constituent phases, one solid and the other liquid, takes place. As the ultramicrons combine, interfaces disappear and all the liquid dispersoid formerly bound to those interfaces becomes free and is exuded from the capillary spaces, whereas the solid assumes greater density. This segregation of phases is known under the name of *syneresis.* The layer of liquid on top of old, "set" fruit jelly, for instance, is a syneretic exudate.

Dehydration and swelling.

5. Just as the autonomous aggregation of ultramicrons causes a loss of capillary liquid, so conversely the artificial withdrawal of water from the system favors aggregation of ultramicrons. The removal of water from a colloid is called *dehydration.* A jelly in air dries out owing to evaporation of capillary water; when soaked in water, it swells again. The amount of water thus taken in depends on a number of physical and chemical factors, but is, at any rate, limited. In organisms loss of water by evaporation can only occur along surfaces in contact with air; these are the exception rather than the rule. Since practically all biocolloids are surrounded by liquids, their dehydration and hydration (swelling) must, therefore, come about in another way than by drying and soaking. Evidently changes in the water

content of biocolloids are due to variations in the ability of the disperse phase to hold water. Any agent raising or lowering this ability becomes thereby a hydrating or dehydrating factor. Potent effects of this kind are exerted by certain ions and enzymes. The immediate effect of dehydration is a condensation of the solid phase.

Fibrillation.

6. The internal structure of a colloid depends upon the shape of its elements as well as on the conditions under which they aggregate during coagulation. The ultramicrons of many biocolloids are *rod-shaped;* this shape may be simply an expression of the elongate, chain-like pattern of the huge protein molecules[1] of which many of the rodlet types of ultramicrons consist. The sol state of these colloids can be likened to a river carrying logs. Ordinary gelation is, in a sense, analogous to a log jam with particles arranged at random; but a more orderly structure can be obtained by forcing the rodlets to become oriented in a single direction. It is obvious that the aggregation of ultramicrons whose axes are nearly parallel must lead to the formation of large units of filamentous character. If the process continues, strands of microscopic *fibrils* and, eventually, macroscopic *fibers* will arise.[2] If the axes of the colloidal particles all lie in the same plane without being parallel, membranous sheets result from their agglomeration. The texture of such fundamental structural elements of the body as the fiber and the membrane is thus revealed to be a matter of the shape of their ultramicrons, plus the action of forces which bring about proper orientation. These forces when acting during coagulation cause the coagulum to turn out with an inner structure of definite orientation; when acting after coagulation they

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[1] The polar structure of ultramicrons has been revealed by polarized light (cf. *W. J. Schmidt*, 1938; see p. 127), the crystalline structure of these units by X-ray analysis (*Astbury*, 1933).

[2] *Nageotte*, 1922, has published a comprehensive account of the physical, chemical and biological aspects of the formation of collagenous fibers of the connective tissue as an example of fibrogenous processes.

may give rise to a reorientation of the structure of the coagulum provided the latter has not yet reached a stage of irrevocable consolidation. The structural arrangement of the ultramicrons in the coagulum will be referred to as *ultrastructure.*

Orientation.

7. Oriented arrangement of rod-shaped ultramicrons can be produced by a variety of agents. When a sol of this kind is allowed to flow swiftly, the ultramicrons are rotated into a common direction, namely, that of the current (compare logs in the bend of a swift river). When *tension* is applied to a gel, the *ultramicrons gradually become oriented along the lines of force.*[1] Electrostatic fields may perhaps yield similar effects.

Ageing as syneresis.

Let us now return to our main subject, the gradual change of the physico-chemical properties of tissues during life leading from a "young" to an "aged" condition and attended by an increase in density and fibrosity as well as by a decrease in hydration and elasticity. One can readily see that these changes correspond exactly to the ordinary life history of a colloid, to its progressive decline of dispersity, accumulation of solid structure and concomitant loss of water. The parallelism is, indeed, so close that one frequently speaks of the "ageing" of colloids. Of course, this might be a mere analogy with no intrinsic meaning, but on the other hand there is some evidence that the resemblance between the "ageing" of colloids and the ageing of tissues is not merely superficial. Extensive tests and comparisons of the colloids of young and of old individuals have demonstrated that they actually undergo a progressive change in the course of life, i.e., decrease in dispersity, hydration, electrical conductivity, elasticity, solubility; and increase in ultramicronic size, stability and electrical resistance.[2]

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[1] *Ambronn* and *Frey*, 1926.

[2] *Růžička*, 1924.

Chemical changes.

The consistent and unidirectional change in the physicochemical — more concisely, colloidal — constitution of the body during development has a corollary on the chemical side. At least it has been stated that if a broad view is taken, a general trend in the chemical composition of the body becomes noticeable. This trend is toward a progressive *cyclization* and *polymerization* of the chemical constituents of living matter.[1] Cyclization means a gradual transformation of open chemical structures into ring-shaped ones, a sort of closing up of the molecules in themselves. Polymerization is the joining together of smaller molecules to build up larger ones. Again, these changes represent merely a general foundation upon which the countless specific chemical reactions and syntheses that occur in the body during a lifetime are superimposed.

DIFFERENTIAL CHANGES

The inequalities of developmental change.

It is obvious that none of the changes thus far discussed could lead to any degree of diversification other than the one present in the egg. If eggs grew uniformly, they would become larger but would remain spherical or ovoid, as the case may be. If their colloidal consistency changed at the same time, again uniformly, they would become larger and tougher eggs with reduced water content but still spherical or ovoid. And even if we add the chemical changes of general nature just mentioned, the result would be beings of larger size, different colloidal consistency and different chemical composition, but they would not be organisms with differentiated parts. It is *differential growth* that transforms an egg into a shape other than spherical; it is *differential physical change* that makes the parts so widely different in consistency and structure as we observe it in the developed body; and it is *differential chemical activity* that lies at the bottom of both differential growth and differential physical changes. In other words, it is not merely the fact that colloidal and electrical and chemical changes do occur, but rather that they occur at different rates with different intensities in different parts of the body at different times that makes development produce the differentiated body. We disregarded this fact when we discussed the growth of the body as if the latter were a homogeneous entity. Now we are ready to supplement the picture by giving our attention to the *differences* of growth through which the constituent parts of the body are distinguished from one another.

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[1] *Pictet*, 1915; *Růžička*, 1929.

DIFFERENTIAL GROWTH

Change of proportions.

When we first compared a baby with a grown-up, our emphasis lay on the difference of size, but upon a second glance, one becomes aware of another difference, namely, the change in *proportions* (Fig. 4). The head of a baby appears much too large and the extremities much too small when compared with those of an adult. Considering the head alone, one

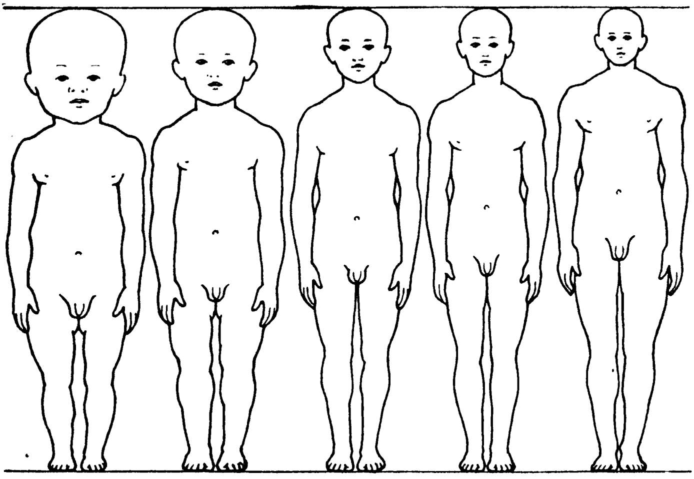

Fig. 4. Change of proportions of the human body during postnatal life. (Modified after *Stratz.*) All five stages reduced to identical size.

finds that the skull is disproportionately large while the facial portion is, relatively speaking, too contracted. As a matter of fact practically every pair of linear measurements which we may care to select for comparison shows distinctive changes not only in absolute dimensions but also in relative proportions. If we had a hollow rubber model of a baby and if we were asked to transform it into the proportions of an adult, we should have to inflate the facial portion more than the skull and the trunk more than the head and the arms and legs more than everything else. One can almost extrapolate from this picture the form of the body as it appears in early embryonic stages: a huge head, mostly skull, crowning a tiny trunk measuring not more than a fraction of the head, and limbs barely visible at all. It takes considerable inequalities of growth to bring a body of this shape to the proportions of an adult.

Growth patterns.

Similar differentials are characteristic of all growth of higher organisms. Every district of the germ has its peculiar growth rate; it is largely owing to this diversity of growth rates that the originally simple egg gradually becomes molded. Differential growth reminds one of a horse race. Though starting from a common base, the field soon scatters according to the different velocities of the individual competitors and reaches the goal in rather complicated distribution. To make the analogy more pertinent, one would have to liken differential growth to a very peculiar field of horses, namely, one whose members always finish in exactly the same order, however often the race may be repeated. This is necessary in order to account for the fact that the ratios between growth rates of different parts are relatively fixed. Otherwise, developed individuals would not typically resemble each other. Growth rates may vary according to circumstances, but so long as their mutual ratios remain constant, they will give rise to similar end forms. Finally, one more correction is needed to adapt the model of the horse race to the realities of growth: in order to express the fact that not all parts in development begin to grow simultaneously, a time handicap of definite length should be assigned to each entrant.

The typical temporal order according to which growth starts in one part after another, and the typical relationship among the growth rates of different parts can be referred to in a simple term as the *growth pattern.*

Heterogonic growth.

The growth of each part, when considered separately, follows the general characteristics of growth outlined above. As for the growth pattern according to which the growth of the constituent parts of the organism is integrated, it varies considerably among different forms. Nevertheless, a few rules applying to growth patterns in general have been discovered; one of these is the law of *heterogonic growth,* revealing a remarkably simple relation between the growth of a given part and that of the rest of the organism.[1] This relation was first established through the study of animals in which one part of the body reaches excessive dimensions as compared with the others: for instance, the claws of the lobster or the antlers of deer. But it was later found to have a wider range of applicability even in less extreme cases.

The heterogonic growth formula.

In order to illustrate heterogonic growth, we chose an example close to the human case from which we started. The change in the cephalic proportions between the juvenile and the adult stage, so striking in man, is still more exaggerated in the monkey or in the sheep dog. Figure 5 illustrates the modification in the monkey. A mere glance at the successive stages reveals that the proportion between the facial and the cranial portion of the head shifts more and more in favor of the former. Just as is the case in man, the face grows faster than the skull. No further regularity beyond this obvious fact appears at first sight. If, however, exact measurements are taken, a definite relationship between facial

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[1] *J. S. Huxley,* 1932.

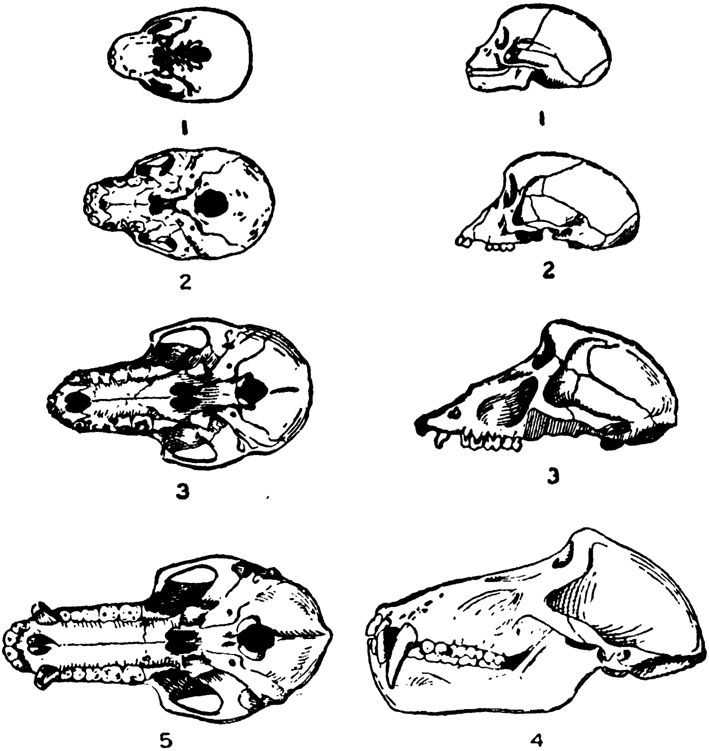

Fig. 5. Heterogonic growth of the face relative to the cranium in the baboon. (After *Zuckermann* from *Huxley.*)
1, new-born; 2, juvenile; 3, adult female; 4, adult male.

and cranial lengths can be recognized. This will be shown here for the case of the dog rather than the monkey because the data on hand for the former are more complete.

If we designate the facial length as y, and total length of the head as x, the relation between them at any given stage has been found to be expressed by the simple formula

$$y = b \cdot x^k,$$

in which b is the facial length at the time when the total length measures unity, and k is an individual constant measuring about 1.5 in the present object. The magnitude of k indicates the amount of discrepancy between the growth progress of the considered part and that of other parts. For objects in which $k = 1$, the formula expresses simple proportional growth; that is, in those cases the ratio between size of one part and size of the others would be the same for all stages:

$$\frac{y}{x} = \text{constant}.$$

For $k > 1$, the formula shows that y (facial length) grows more rapidly than x (cranial length). In the case of $k = 2$, for instance, facial length would have increased four times when cranial length would have doubled, and nine times by the time the latter had trebled. If k is below unity, this simply means that y grows more slowly than x.

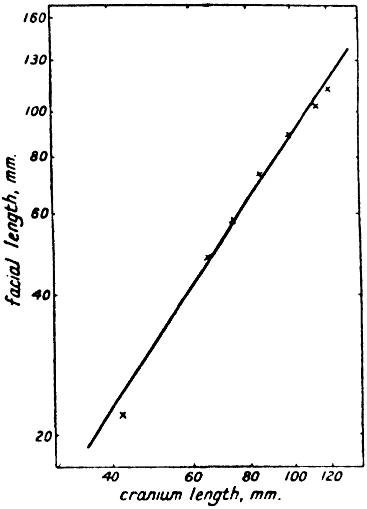

Fig. 6. Heterogonic growth of the facial region relative to the cranial region of the skull of the sheep dog. (After *Becher* from *Huxley*.) Abscissae and ordinates in logarithmic units.

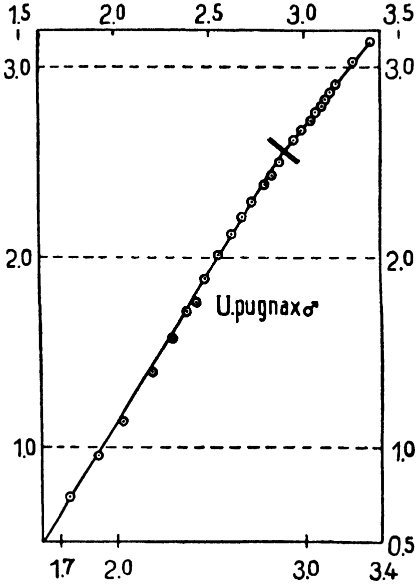

Fig. 7. Heterogonic growth of chelae in male fiddler crabs. (From *Huxley*.) Abscissae: logarithms of body weight. Ordinates: logarithms of weight of chelae. The resulting curve is a straight line with an inflection point.

The above formula can also be written as follows:

$$\text{Log } y = k \cdot \log x + \log b.$$

In other words, if the logarithms of facial length are plotted against the logarithms of cranial length, the resulting curve is a straight line with k the tangent of its angle of incidence (Fig. 6). The data plotted here for the sheep dog show how closely the formula fits the actual facts. In order to stress the fundamental character of the relation we reproduce still another example from an entirely different group. Figure 7 shows the same formula as it applies to the size of a claw in relation to the weight of the body in male fiddler crabs of a variety of ages. As the body grows, the claw grows, but the latter grows much faster than the former, so that the proportions of the animal change constantly. One is reminded of the rapidity with which the offshoot of a tree grows as against the slow enlargement of the main stem. Yet in spite of the fact that the growth of the claw and that of the rest of the body are to all external appearances out of step, the formula reveals that they are strictly linked up with each other.

Interpretation of heterogonic growth.

Here, then, is a formula applying not to the growth of the individual parts as such, but to their mutual relationship; in other words, to the *growth pattern*. The formula is an empirical one, an abbreviated representation of simple facts. But what does it actually mean?

In the first place one notes that the formula pertains immediately to achieved *size* rather than to growth. Time never appears in it. The size of a part at any given stage is expressed in terms of the size of the body, irrespective of how and in what time this size was attained. This appears so only on the surface, however. For, as can be shown by a simple mathematical transformation,[1] the heterogonic for-

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[1] Differentiating the heterogonic function

$$y = b \cdot x^k, \tag{1}$$

one obtains:

$$\frac{dy}{dt} = k \cdot b \cdot x^{k-1} \cdot \frac{dx}{dt}; \tag{2}$$

since, according to (1),

$$b \cdot x^{k-1} = \frac{y}{x}, \tag{3}$$

equation (2) can also be written:

mula does, in effect, refer to growth rates. Specifically, it implies that the *ratio* between the growth rates of parts y and x remains *constant* over the whole period for which the formula is valid; not the rates, but their ratios remain constant. If, for instance, the part y grows half as much faster than the rest of the body x during a certain early period, it will at a later period still be found to grow half as much faster than the rest of the body, although the absolute growth rates of both have markedly declined in the meantime. The relation is as that between two sums of capital invested at different interest rates; although one will accrue profit at a higher rate than the other, the proportion of their individual rates of increment will remain constant. If the ratio becomes unity, both capital sums grow at an equal pace, and in the case of growth this means proportional growth.

Although the heterogonic growth formula is unquestionably of great usefulness and wide applicability, one must remain aware of its limitations. In the first place it represents the actual situation only with a certain approximation; this is more or less true of all attempts to reduce complex biological phenomena to mathematical terms. Secondly, it lends itself to ready verification only in cases in which an organ of exceptional growth rate is contrasted with a body of otherwise fairly uniform growth; including those cases in which there is a steady falling off of growth rates along the axis of the body — a cephalo-caudal growth gradient

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(4) $$\frac{dy}{dt} = k \cdot \frac{y}{x} \cdot \frac{dx}{dt},$$

or

(5) $$\frac{dy}{y \cdot dt} = k \cdot \frac{dx}{x \cdot dt}.$$

Since, according to the definitions on page 16, $\frac{dy}{y \cdot dt}$ and $\frac{dx}{x \cdot dt}$ are the relative growth rates V_r of the masses y and x, respectively, it results that

(6) $$V_{ry} = k \cdot V_{rx},$$

and therefore,

(7) $$\frac{V_{ry}}{V_{rx}} = \text{const.}$$

— which is a rather common occurrence. But the more irregular the distribution of partial growth rates in the body becomes, the less accurate becomes the heterogonic formula. Thirdly, even in instances in which the formula fits the facts very nicely, the growth parameters remain approximately constant only for certain periods and then change more or less suddenly. This can be seen from the marked inflections which the growth curves incur at certain points (cf. Figs. 6, 7); many growth curves appear broken up into several fragments which cannot be fitted into a common curve. There are still further complications which fall outside the scope of this survey.[1]

Although, on the whole, we seem to be still far from possessing a picture of growth that could be considered as of general validity, one should not underrate the invaluable contributions to our conception of development which have come from studies of growth. It is largely to their credit that we have ceased thinking of an organism in the rigid static terms gained from anatomical studies, and have acquired instead the habit of regarding any morphological stage as merely a fixed expression of the degree of progress and perfection which the various developmental *processes* had reached at that time. One has learned to emphasize the dynamics of the happenings rather than the static results or stages of their accomplishment. Products which, from the static viewpoint of the morphologist, look so different as the skulls of young and old baboons (Fig. 5) are now seen to be identical so far as the dynamics of their making are concerned. They are samples, as it were, taken at earlier and later stages of the same head-forming process. In other words, two organisms which started with the same growth formula, i.e., the same distribution and ratio of growth rates, but were arrested at different times in their development, can be quite dissimilar in shape and certainly lack geometrical proportionality. Consequently, it would be unwarranted to consider two organisms of strikingly different proportions as of different growth pattern just because of their unlike appearance. And our classification and comparison of organisms must rest on the characteristics of the whole course of the developmental curve rather than on the characteristics of the end point which is often accidental; on the general *law of growth* rather than on its varying individual manifestation.[1]

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[1] See the discussions of *Schmalhausen*, 1930; *Glaser*, 1938; *Robertson*, 1923.

Different parts of the body start to grow at different times.

The range in which heterogonic formulae are most nearly correct is that of the *post-embryonic* period, when the body has its full set of organs. The further we go back in embryonic history, the less readily can the realities of growth be described in simple formulae. The main reason for this lies in the initial lag of various organs with regard to one another. Different organs date their origin from different periods of the embryonic history. Some are older, others are younger; the limb appears later than the eye, and the kidney later than the limb. These age differences among various organs which become indiscernible in later life are of course quite conspicuous in the growth pattern of the early organism. An age difference of one month means nothing in an old man, but it means very much in a baby. Consequently, the early growth pattern is determined not only by the distribution of growth rates but just as significantly by the *time sequence in which partial growth processes start.*

Growth depressions.

Another obstacle to simple mathematical treatment of growth during embryogenesis is its lack of continuity; for, embryonic growth advances unevenly, in spurts and jumps, with intermittent depressions. These depressions, as will be shown later, correspond to phases of intensive histological differentiation, but all that is of concern in the present context is that they do occur and disrupt the smoothness of the growth curve (cf. Fig. 18).

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[1] See *D'Arcy Thompson*, 1917.

Differential dependence of growth rate upon environmental conditions.

In order to leave no doubt about the complexity of the growth problem, it seems appropriate to point out that, in addition to the differential effects attributable to the constitutional growth patterns, lack of uniformity in the response of growing systems to the environmental conditions accounts for further distortion of proportions in the course of development. The effect of a change in the temperature level may be quoted as an example. As stated above, growth rates go up as the temperature level is raised. Since the basic temperature coefficient is nearly the same for all growth processes, one would expect that the growth of all parts would be speeded up proportionately so that the body would merely grow faster while keeping its typical proportions. This is not invariably true, however. There are observations to indicate that a higher temperature level does not necessarily produce uniform acceleration of growth but often favors some parts to the disadvantage of others and thus distorts the proportions of the growth pattern.

Quite generally, growth in higher temperatures leads to forms with longer appendages and a more jagged contour, whereas lower temperatures produce more compact forms with plumper and shorter appendages and with a simpler and smoother contour. Higher temperature promotes the growth of limbs, tails, gills, etc., more than that of the bulk of the body; the snouts, legs and tails of rats and mice raised in warmth are not only absolutely but also relatively longer in proportion to the body than those of specimens raised in the cold.[1] Figure 8 illustrates the differences in the development of the gills of amphibian larvae reared in different temperatures; the higher the temperature, the more numerous, ramified and slender are the gill filaments. In other words, expediting growth in general benefits certain parts more than others. And noting that the greatest share goes to the appendages which already excel by their high relative growth rates, one is led to the conclusion that *parts with intrinsically higher growth tendencies derive a greater enhancement from a general speeding-up* than do the less active parts. The explanation for this behavior is to be sought not in local differences of temperature-dependence of growth but in interactions among the growing parts themselves. Inasmuch as the matter is illustrative of a whole class of mutual influences among parts of the developing organism, it deserves further elucidation which we shall attempt with the aid of a simple model.

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[1] *Sumner*, 1915; *Przibram*, 1925. There is a striking parallelism between the morphological differences among individuals raised at different temperatures on one hand, and species growing in different climates on the other. Of two closely related species of which one lives in a cold and the other in a warm climate, the latter usually possesses appendages which are longer in proportion to the body than are those of the former (see *Hesse*).

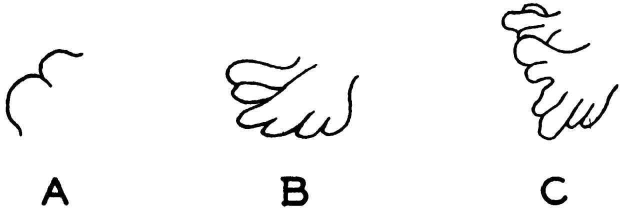

FIG. 8. Effect of temperature on morphological characters. Gills of frog tadpoles raised at three different temperature levels, drawn at comparable stages of development. (From *Doms*, 1916.)
A, 15° C. B, 23° C. C, 33° C.
Note the increase in number and length of ramifications with increasing temperature.

A model of unequal growth promotion.

When a drop of liquid falls on an even surface, the impact causes the particles to spread centrifugally at a rate which is in some proportion to the height of the fall. By increasing this height, one increases the speed with which the splotch forms after the impact, and consequently also the size to which it grows. The important point about this enlargement, however, is that it is not uniform, although quite regular. Let drops of ink fall upon paper from various heights (Fig. 9). The drop falling from the lowest height (a) forms a round spot smooth in outline. A drop from the next higher level (b) is larger in diameter and its contour is wavy.

Coming from still greater height (c) the drop displays distinct lobular processes along its circumference, and finally, if we increase the height of the fall still more (d), we observe that these processes become more numerous, slender and longer. This series represents the morphological results of the "growth" of ink spots at different rates. It clearly illustrates that gradual change of speed does not only affect the *magnitude* of the result but also its essential *morphology*. The transition from the massive, smoothly outlined shape obtained at low speed to the star-like shape with its jagged contour at great speed imitates very closely the corresponding series of morphogenetic variations of organisms which have grown at different rates.

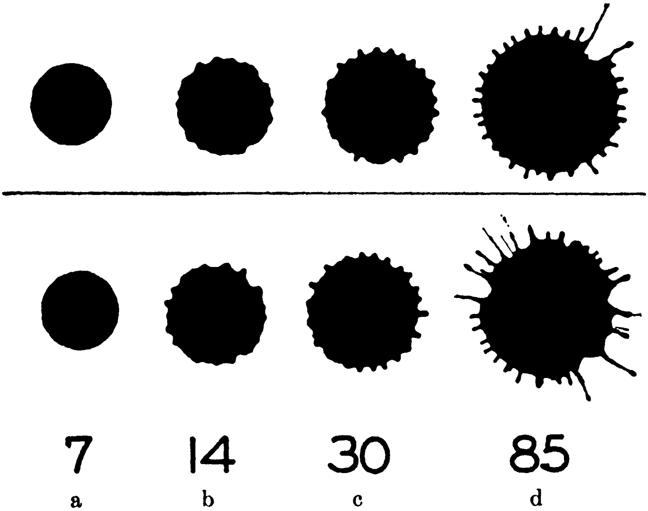

Fig. 9. Morphology of ink spots produced by drops falling from different heights. Figures indicate height in cm. Increasing height of fall, that is, increased speed of spread or "growth," produces increase in diameter of spot, increase in number of protrusions, and increase in length of protrusions.

Now for the explanation: The contour of the spreading ink spot is a compromise between antagonistic forces; the force of the impact tends to disperse the mass centrifugally but forces of cohesion, surface tension, and elasticity resist and hold the mass together. Expansion is possible only to the extent to which, and as long as, the former overbalance the latter. The greater the speed, the farther the former extend before being checked by the latter. From a certain speed upward, the restrictive forces can no longer hold up the swift advance over the entire circumference. As a result, the expansive drive breaks through the front in certain weak spots which are due to random inequalities in the conditions of paper, ink, and impact. This opens gaps, as it were, in the line of resistance, and through these the ink flows out in a number of swiftly progressing columns. This has an important consequence; each local flow, once established, sucks in substance from the vicinity so that the central mass of ink keeps feeding into the various existing protrusions instead of establishing new ones. Thus the very act of producing a local outgrowth inhibits the production of a similar outgrowth within a certain range by directing all available substance and motion into the once established channel. The greater the velocity and the more vigorous the progress of the local outgrowth, the greater becomes the drain into it from the vicinity; the greater, therefore, are the inequalities of growth along the contour. This is the reason why the gain from a general speeding up goes primarily to those processes which have already an initial advantage.

"Dominance" of more active parts.

This story of the "growing" ink spot illustrates a very general principle of morphogenesis. Whether one is dealing with growth or with organ formation or with regeneration, local progress when once established tends to persist and to suppress by this very fact the manifestation of similar tendencies within its vicinity. The phenomena of "dominance" and "subordination" in development which are frequently described with reference to so-called metabolic gradients and which will be discussed on a later occasion (p. 380) are merely special cases of this general natural principle according to which those parts of a system which have greater momentum gather strength from the rest. And to return to our present subject, this is also the reason why a general increase of growth intensity, for instance, by raising the temperature level, must benefit the faster growing processes to a greater extent than the bulk of the body whose growth is partly inhibited by the progress of the former. Whether the depressing effect of an actively pushing growth center upon its surroundings is due to an actual withdrawal of chemical substances or of physical conditions prerequisite for growth is of no concern in the present context.

The direction of growth.

In the preceding pages a few fundamental properties of growth have been outlined which make comprehensible how an organism attains shape and why the proportions of its parts shift as it grows. But we have accounted only for *intensity* and *rate* of growth and not yet for the *direction* in which it occurs. A brief consideration of this point will show its importance.

The simplest type of growth is three-dimensional growth in which equal amounts of mass are added in all three directions of space. Geometrically this produces strictly proportional enlargement such as is rarely observed in multicellular organisms. It is more nearly realized in the growth of individual cells. The direction in which an organ grows is determined not only by the direction in which the individual cells expand, but also by the orientation in which the new cells separate in the act of division as well as by the arrangement in which they become joined to form higher units of tissue. In other words, differential growth (in the sense of pure increase of living mass) tells only part of the story of organic shape. The other part is furnished by extrinsic conditions forcing the newly produced cells into definite spatial configurations. These conditions control the geometrical rather than the arithmetical aspects of growth. Depending on the attending conditions, growth can, therefore, produce not only three-dimensional enlargement but one- or two-dimensional expansion as well.

One-dimensional, or linear, growth is present when all the new materials are added strictly along one line. This is exemplified by the early stages of mesoderm formation in Annelids (see Fig. 88) leading to the production of the so-called mesoderm streaks; also by the outgrowth of the lateral line system of lower vertebrates (see Fig. 28). The lengthwise growth of hollow ducts and solid cords belongs essentially in the same class. The two-dimensional type of growth results in the formation of cellular layers or sheets or membranes called *epithelia,* irrespective of the kind of cells constituting them.

Morphogenetic role of directed growth.

What particular type of growth is to be followed in a given case, is determined partly by the character of the cells, partly by the condition of their immediate environment. This will be discussed more fully on a later occasion. For the time being, however, it is well to keep in mind that the geometry and arithmetic of growth are controlled by separate factors. The following simple example may serve to indicate the implications of this fact for the shape of growing organs. Let us visualize two cell masses in close apposition, growing at the same rate, but one — E (for epithelium) — with a tendency to two-dimensional growth, the other — M (for mesenchyme) — bound to expand three-dimensionally. Let us assume further that at the beginning of our observations the layer E smoothly covers the underlying mass M. Then we wait until both have grown to eight times their initial mass; owing to the assumed equality of growth rates, both reach this stage at the same time. What shape do they have now? At the end of the period the portion M which has grown evenly shows a uniform increment of 2 ($= \sqrt[3]{8}$) in all directions. Its surface has, accordingly, increased 4 ($= 2^2$) times. At the same time, however, the surface of the fragment E with two-dimensional growth has increased just as much as its mass has, that is 8 times. In other words, the covering epithelium E has expanded precisely twice as much as that portion of the surface of M which it had covered in the beginning. The physical expression of this discrepancy varies depending on where the two portions are fused. If contact between E and M is maintained throughout, the more rapidly expanding layer E overgrows the more slowly expanding substratum M, or if E becomes detached from M in certain places, it bulges out or folds in. In any case, *shifts of the cellular masses relative to each other* must necessarily result from the incongruous expansion of the two surfaces.

Important architectural features of the body such as folds, grooves, ridges, fissures, and all sorts of distortions can arise in this manner merely owing to the different geometry of growth of neighboring tissues, even though their growth rates may not differ at all. Considering this fact in conjunction with the other one, that growth rates actually do differ locally, one becomes aware of the inadequacy of a purely arithmetical treatment of growth. Growth formulae, including those of heterogonic growth, should, therefore, be applied with reason; there is no point in stretching them beyond the limits within which complex situations can be reproduced by simple mathematical operations.

Our discussion has been drifting gradually from the contemplation of pure growth to differences in the specific character of growing parts through which their increase is checked and directed into definite patterns. This brings into view the second major phenomenon of development — *differentiation.*

DIFFERENTIATION

The increase of diversity during development.

Even without special powers of observation one can recognize that a developed body differs from its own earlier stages not only by its greater size, changed proportions and different general consistency, but just as much by the specialized aspects which the structures and functions of its constituent parts have assumed. There are *more* parts of specialized function present in the end than there were in the beginning of development, and they have increased in *diversity* as well as in number. This experience, gained from superficial observation, has been amplified by finer studies of the development of structure and function. Progressive diversification is acknowledged as the keynote of development. The whole complex of changes involved in this diversification is commonly referred to as *differentiation.*

What is differentiation?

There is often a deplorable lack of precision in the usage of the term "differentiation." Endless arguments have arisen from the fact that different people had quite different things in mind when talking of differentiation. Although such vagueness of expression does not generally interfere with the execution of an experiment, it certainly may prevent the correct appreciation of its results. In order to be more explicit in the use of the term "differentiation," we propose to sort out the numerous phenomena customarily subsumed under it. As will be seen, they are heterogeneous and disparate phenomena.

The fundamental plan of the organism.

Every higher organism shows certain geometrical regularities which can be used to characterize its basic pattern. The main features are *symmetry*, *polarity*, and the *repetition* of structures in a definite spatial order. In most forms one can locate at least one plane with regard to which an animal is essentially symmetrical (bilateral symmetry). In some forms there are two (bisymmetry) or even more (radial symmetry) such planes. That plane of symmetry which includes the front and rear ends and cuts through the middle of the dorsum and ventrum is called the *median plane.* The linear dimension of this plane connecting the front end with the rear is called the antero-posterior axis, while any line perpendicular to it and running in the dorso-ventral direction can be designated as dorso-ventral axis. It must be kept in mind that axes are not actual lines in definite locations but merely convenient symbols to indicate general directions. If the structure of a body changes as one progresses along one of its axes, this fact is described as *polarity.* For instance, polarity with regard to the antero-posterior axis means that the front portion of the body is different from the rear portion. Planes laid at right angles through different points of the main axis are referred to as *levels* of the body. *Segmentation* is the repetition of structural patterns in successive levels of regular spacing. These characters indicate the fundamental plan of the organism.

The Organs

Organs and systems.

Fitted into the fundamental plan of the organism are *organs* arranged and distributed in a definite order. Some of the organs are clearly circumscribed and outlined on practically all sides, as for instance, the eye, the liver, the kidney. Others are only well outlined on one side but ill-defined on the other — comparable to a relief sculpture; examples of this group are the limb and the nose. Others are still more vaguely defined. But all these organs are distinct and localized. That is, they are inserted in a certain spot of the body where they occupy a certain amount of space. In addition to the discrete organs there are *systems* of greater continuity, more ubiquitous in occurrence and pervading the body more diffusely — the peripheral nervous system and the various vascular systems (blood vascular, lymph vascular). Each of these particular structures has its special function, form, distribution, arrangement, and connections. All of them are embedded in, and held together by, a matrix of less specialized connective tissues which support, connect, and protect the enclosed organs and systems. And finally, in addition to these solid constituents, there are liquids, some belonging to the body and circulating in it (blood, lymph, cerebro-spinal fluid, peritoneal fluid), others ready for discharge into the external medium and which can no longer be counted as genuine constituents of the body (urine).

Most organs are *complex;* that is, a variety of tissues take part in their construction. Even where the specific functional part of an organ is of uniform construction, as for instance, in the case of nerves or muscles or glands, the association between the specific elements (nerve fibers, muscle fibers, secretory cells) and the surrounding matrix is generally so close that such organs must again be considered as composite in character. All organs have in common certain elementary vital functions, namely, *metabolism*, *respiration*, and *excretion*. In addition to these general functions, each organ displays *specific* functions and appropriate structures to serve them. The gut digests, the muscle contracts, the gland secretes, the nerve conducts.

Functional properties of organs.

1. With regard to *function*, the following properties of organs are noteworthy.

a. Location, arrangement, structure, internal organization, connections, vascularization, innervation, etc., are adapted to the specific tasks which the various organs are called upon to perform. A muscle is not merely a mass of elements ready to contract when stimulated. Its function is contingent on much finer adjustments. Muscle fibers which move parts are different from those which serve to hold them in position; their length and elasticity are so balanced as to prevent overstretching without hindering the free movements of the joints; their vascularity is so adapted as to insure sufficient blood supply even at the height of activity; and so forth. And a muscle is a rather simple organ. In a more complicated organ, like the eye, the mere enumeration of the prerequisites to its orderly functioning would fill many pages. Any text on the physiology and psychology of vision conveys a rough idea of the perplexing number of conditions which must be satisfied in order that we may not only discriminate light from darkness but actually see patterns, colors, forms, and movements. Therefore, when thinking of an eye, remember always this wealth of specific properties of which the anatomical and histological picture reveals a mere fraction.

b. The organs of the body do not function as independent individuals but maintain constant interrelationships; this interaction is provided for by special devices. Many organs are correlated among each other in the form of functional chains; the action of one releases actions of others which in turn may act back on the first and modify or check the original activity. Thus, the nerve starts the muscle going, the hypophysis through its secretion stimulates other glands of the body, and so forth. The main point is that the links of the various chains must fit into each other in a very specific way to permit smooth functioning of the organism. A nerve fiber must possess very specific properties in order to be able to excite a muscle fiber of the type with which it connects. The visual cells of the eye must be equipped with just such a physical and chemical mechanism as will respond to the wave lengths of the visible spectrum which are of biological significance. Thus the functional equipment of an organ provides not only for its own functional capacity but at the same time for its functional relations to other organs as well as to the environment. This includes provisions for the coördination of various organs in the aim of producing coöperative effects — so called *integration.*[1]

The composition of organs.

2. From the *structural* viewpoint, each organ appears composed of *tissues* which determine its "texture."[2] One distinguishes four fundamental types of tissues: (1) epithelium, (2) muscle tissue, (3) nerve tissue, (4) connective substance (including blood, cartilage, bone, and connective tissue proper). The tissues, in turn, consist of cells, intercellular substance (matrix) and tissue spaces.

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[1] As a safeguard against the danger of an oversimplified concept of the organism one should constantly bear in mind the fact that the mutual interplay among different organs is still infinitely more subtle and intricate than are the functions of the various organs themselves, when taken individually. Cf. *Cannon,* 1932.

[2] It has been anticipated in this book that the reader has some elementary knowledge of Microscopic Anatomy. For more advanced information, see the textbooks of Histology, e.g., *Maximow* and *Bloom,* 1938.

a. The *specific* cell types are the ones to which an organ owes its peculiar function. For example: the visual cells of the retina responsive to light; the secretory cells of a gland producing and discharging specific substances, hormones, or enzymes; the hematopoietic cells of the bone marrow; the contractile fiber syncytia of the muscle.

b. The *accessory* cells are functionally largely subsidiary to the former, in that they provide nutrition, support, and protection. To this class belong glia cells of the nervous system, Muellerian fibers of the retina, follicular cells of the ovary, sheath cells of Schwann around the nerve fibers, cells of the sclerotic coat of the eye, and finally most of the connective tissue cells pervading the organs and covering their surfaces.

c. The *reserve* cells, while ordinarily exhibiting none of the specialized characters of group (a) or (b), are yet known to be able to turn into either one or both of these groups in certain circumstances. They are cells that have been arrested in their development to serve as reserves upon which the organ may draw in the case of necessity. This necessity arises either in the course of regular physiological destruction owing to wear, or from accidental destruction, in which case reserve cells assume the role of regeneration cells (see also p. 468). The duct cells of most glands, for instance, belong in this class.

d. The non-cellular *matrix* in which the cells lie embedded is a product of cells, which has arisen either through the transformation of cell bodies or parts of such, or by secretion of non-living substance into the intercellular spaces. It is ubiquitous but its character, composition, and relative abundance vary locally. Although it fails to exhibit the vital functions of metabolism, respiration, and assimilation, which are confined to the cell bodies, its functions are so important and indispensable for the maintenance of the organism that it must be considered as an integral part of the living system. There are a number of organs whose bulk consists of non-cellular matrix while the cellular content represents a minor fraction only of the total mass. Such organs are the lens, the vitreous body of the eye, bone, tendons, and many other connective tissue formations.

e. The *tissue spaces* extend into the matrix and pervade it in a ramified or anastomosing network. They are filled either with tissue juice or with branches of the diffuse conduction and circulation systems of the body — nerves, blood and lymph vessels. The average size and density of these branches as well as the pattern of their distribution vary characteristically for the different organs. The nerves come from definite sources in a definite distribution and form definite connections appropriate to the organ (for further details see Part IV). Similarly, the size, proportion, and position of arterio-venal connections in the capillary network as well as the whole pattern of the vascular tree vary for different organs and are as finely adapted to the function and vital requirements of each type of organ as the fuel and oil flow is adjusted to the consumption requirements of an engine.

From this survey of the general composition and setting of the organism we pass on to outline briefly the properties of its constituents — the cells and their matrix. The list is incomplete but will suggest the diversity of phenomena which must be taken into consideration if the term "differentiation" is to become more than a vague phrase.

The Individual Cells

1. *Shape.* Highly specialized cells with special structural equipment (e.g., sensory cells, muscle fibers, unicellular organs) can be identified by their very definite shapes. Other cell types exhibit a much wider range of morphological variability. The cell of an epithelium may be flat or columnar. A connective tissue cell may be star-shaped and plump, or very slender and spindle-shaped. Even nerve cells, while rather well-defined in their form, vary within

wide limits with regard to number, length, and ramification of their processes. Sensory cells, such as the hair cells of the inner ear and the rods and cones of the retina, represent the extreme of morphological constancy.

2. *Size.* Each cell type falls into a particular size class with comparatively slight fluctuations. Some cell types, however, appear in more than one size category. In many cell forms the size varies, furthermore, in accordance with the state of functional activity, hormone level, nutrient milieu, and the like (compare Fig. 14).

3. *Functional equipment.* This includes all the peculiar devices with which a cell is provided in the service of its specialized function, such as the different chemical apparatus through which such specific secretions as digestive enzymes, hormones, acids, mucus, fibers, etc., can be manufactured, contractile apparatus of muscle fibers, the fibrils and the neuroplasmic apparatus underlying conduction of excitations in a nerve fiber, the apparatus transforming light energy into chemical processes capable of producing visual nerve impulses, and so forth.

4. *Reactive properties.* No less characteristic than its active function is the specific sensibility and responsiveness of a cell toward certain agents. The sensitivity of a given visual cell for a narrow band of the light spectrum is only one example of many. Selective reactivity of some kind is one of the most fundamental properties of cells. A mammary gland cell reacts selectively to the stimulus of lactation hormones; a sympathetic nerve ending reacts selectively to adrenalin; a thyroid cell reacts selectively to the presence of iodine in the body fluids; "selectively" meaning that although all kinds of cells are exposed to those agents — light, hormone, adrenalin and iodine, respectively — in each case only the specifically predisposed cell types show a response.[1]

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[1] The selectivity frequently is not absolute but relative in that some cell types merely have lower thresholds of reactivity toward given kinds of stimuli than others. Under weak stimulation only the most susceptible cells will react, whereas strong stimuli will produce a general response.

5. *Mobility.* Some cells have a permanent domicile while others are more or less perpetually on the move. Indeed, with some, movement is part of their function. Blood cells, germ cells, pigment cells, and phagocytes belong in this class. Even epithelial cells of the skin and endothelial cells of blood vessels and fixed connective tissue cells may be caused to migrate by certain stimuli.[1] The degree of mobility as well as the mechanism and the conditions under which the motion is executed are distinctive for different cell types.

6. *Mitotic rate.* The capacity of cells to divide and proliferate varies considerably among the different tissues. In general it can be said that, the more specialized a cell is in its structure and function, the less apt is it to divide. Mitotic ability seems thus to be limited more by the degree than by the type of specialization. On the other hand, mitotic rate, the rhythm of cellular multiplication, is again distinctly different in different tissues of the body; this fact could be inferred from the occurrence of differential growth (see p. 36).

To sum up: the reactivity of a cell, its mobility, its capacity to grow at a certain rate and to divide after it has reached a typical size limit — all these characters belong to the constitutional properties of a given cell type just as much as do the more obvious morphological and functional characters. It cannot be emphasized too strongly that our notion of the cell must never confine itself to the conspicuous visible structures and patent functions, but must embrace the sum of distinctive properties and capacities.

Let us now turn our attention from the individual cell to the cell group. After all, every organ of a higher organism is a complex community of cells, and new properties emerge from this integration.

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[1] Commonly in response to pathological irritations, such as injury, inflammation, infection.

The Cell Communities

1. *Cell numbers.* In some lower invertebrate groups of microscopic size the number of cells building up an individual is constant.[1] This is not found to occur in higher and larger forms although even there individual organs sometimes come very near to possessing a constant content of cells. Since, as mentioned before, the size of individual cells keeps within definite bounds, it follows that in those organisms whose final size is limited (see above p. 17), the total number of cells, which is roughly proportional to the quotient of total mass over cell size, is normalized. The same holds then for the partial numbers of cells constituting a given organ. Generally, the smaller an organ, the less is the variation in the numbers of cells composing it. Also, the more specialized organs tend toward greater cell constancy. Not only the absolute numbers of cells present in an organ but also the relative proportion in which different categories of cells are represented in the organ keep within a definite norm. For instance, the ratio between rods and cones in the retina of a vertebrate eye is fairly constant for a given species. The same is true of the ratio between motor horn cells and other nerve cells in the spinal cord. Even when large numbers of cells are involved, as in the spinal ganglia for which we have exact counts (p. 550 f.), variation among individuals does not exceed relatively narrow limits. Whether the number of cells in an organ is controlled by a limited capacity of the individual cells to produce progeny or by restrictions on the part of the cell community, is a question to be discussed later (see p. 423).

2. *Cell density.* Cell density is subject to distinct local variations. Of course, many of the factors mentioned in the preceding points such as cell size, mitotic rate, resulting number of cells, production of intercellular substances, etc.,

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[1] Cf. *Van Cleave*, 1932; also *Tabulae Biologicae*, edited by *Oppenheimer and Pincussen*, vol. 4, 1927; table on p. 306.

have a bearing on it. In addition to these, however, factors of clearly collective origin enter the picture, such as mutual attraction (aggregation) or repulsion (dispersion) of cells, prevention of expansion through spatial restrictions set up by other cells, and the like. See also the next point.

3. *Cell pattern.* The spatial arrangement of cells into groups is again partly dependent upon their individual size, shape, mode of proliferation, and so forth; in other words, upon the properties of the individual elements. In a larger measure, however, the spatial arrangement is imposed upon the individual cells by conditions which are the resultant of collective activities. The stratification of the brain (p. 509) or of the retina (Fig. 11), the definite grouping of cells in cartilage, bone (p. 454), connective tissue (p. 448 and Fig. 97) — all these patterns result from conditions prevailing in the organ as a whole rather than from independent activities of its member cells. Definite spatial arrangements can also arise through primarily oriented growth (see p. 49), but more commonly they are brought about through secondary regrouping and realignment of cells which were formerly less definitely oriented. Looking, for instance, at the cross-section of a notochord (Fig. 49, p. 255), one observes a perfectly circular outline and a rather irregular pattern of cell boundaries on the inside. It is obvious that these cells have not assumed their odd forms independently to be put together afterwards, as one sets a pavement from stones hewn to match; it is the inner turgor that makes the whole mass assume cylindrical shape in the first place, and then each individual cell bubble has to accommodate itself as best it can in accordance with this established form of the whole mass. This point will be taken up more fully on later occasions.

4. *Cell affinities.* Different cell types have different preferences, as it were. Sheath cells join with nerve fibers, pigment cells follow connective tissue strands, primary sex cells move into the gonad, almost like parasites which select

preferential locations of the host body to settle there. Naturally, such differential affinities are instrumental in producing and maintaining the heterogeneous composition of complex organs.

5. *Matrix.* This is the medium through which the cells are interconnected. Being a product of cells as well as constantly under their influence, it can be expected to show a similar range of diverse characters as do the cells themselves. This is certainly true inasmuch as the matrix varies in regard to consistency, physico-chemical and chemical character, architecture, whether it is fibrillated or laminated or spongy, whether or not it can be digested by certain enzymes, whether or not it accepts certain histological stains, whether and what sort of minerals are deposited in it, etc. As a matter of fact these specific properties of matrices might rightly be added to the account of those cells from which they have originated.

Thus the list of the peculiar faculties and characters with which every cell is endowed assumes rather impressive dimensions. The list is far from complete, but it gives a rough idea of the degree of diversification to be found in a developed organism. Adding all the minor distinctions that exist between cells of the adult organism would swell the list to the size of a voluminous catalogue. For our purpose it is sufficient to keep in mind that such tremendous diversity exists in addition to the elementary vital functions which are common to all cells. When we say diversity, we mean *typical* diversity; that is, we refer not merely to the fact that there are differences but that the various distinctive and specific characters are distributed through the organism in a definite *order*, *pattern*, and *proportion*, numerically and topographically well-defined. This is what one calls *organization*, and since it can be revealed directly either by observation or by critical experimental tests, we may call it *manifest organization*. The adult organism has a maximum of it (see p. 103).

The Development of Organ Characters

Tracing back development.

Now, let us take a broad jump and go far back in the history of the individual — back, indeed, to the point where the individual for the first time deserves this name. There we find a fertilized or unfertilized egg, and if we disregard for the moment the difference in size, the first thing to strike us is the extreme paucity of recognizable differentiated characters and the low degree of manifest organization. The egg is not only smaller but it is incomparably simpler in its outfit than the developed organism. However, all we can gain from this comparison of the two end points of the developmental line is a general and commonplace statement to the effect that somehow, somewhere along the line the increase in complexity and diversity must have arisen. If we wish to obtain more specific information about the how and when, we must trace the events through their whole course. Since we know the developed organism much better than we know the egg, it seems more expedient to trace our steps back from the former than to try to reconstruct them in the ascending order. Accordingly, our procedure will be to trace each item of our list back as if we possessed a moving picture of their development which we now unreel in the reverse direction. In this sense, wherever in the following we say that a certain character ceases to be present, this means in the actual order of embryological events that it has just made its appearance.

Tracing function.

1. The first thing to disappear from our backward records is *specific function.* Following a gland backward, we come to a point where it does not secrete; tracing a muscle, to a point where it cannot contract. While it is comparatively easy to make definite statements about the inception of the heart beat, muscle contraction, and gland secretion, all of which have observable effects, it is much more difficult to

ascertain at just what time an eye acquires the ability to see or an ear the ability to hear; we can merely surmise that these functions have a similar kind of embryonic history as the recordable motor functions. (More about functional development on pp. 558–573.)

2. The cessation of function, as we go back, is only partly due to obvious functional shortcomings of the organs themselves. In a measure it is due to the *absence of correlating systems*. We come to a point where nerves, though present, have not yet formed functional connections with their end-organs, and further back there is a point where the nerves are not even present. Similarly, the blood-vascular system appears the less complete, the further we retrace its steps. As the large communication systems of the body, the nervous and the humoral, disappear as we go back, the direct and rapid exchange of messages and substances between the organs also ceases.

Tracing shape.

3. The *individuality* of organs becomes less distinct as we proceed backwards. We come to a point where the organs are no longer sharply set off from each other. Membranes, sheaths, capsules, and other devices which later mark off individual organs against their surroundings have not yet been formed. Consequently, the various organs are also held more firmly in position.

4. The *shape* of the organs becomes increasingly *simple*. Partly this is an expression of differential growth. The contribution of differential growth to the complication of form has been discussed above (p. 46), and there is no need to return to this point. The development of the limb (Fig. 10) or heart may serve as an illustration. A hand in an earlier phase has the shape of a mitten, still earlier of a paddle, and earlier still is a shapeless bulge. The heart at an early stage is represented merely by four widenings of a tubular structure wound up in a coil and representing the bulb, ventricle, auricle, and sinus, respectively. Still further back the tube

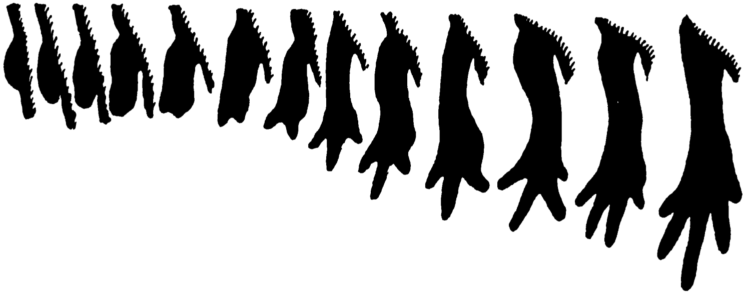

FIG. 10. Successive stages in the development of the left hind limb of the newt, *Triton taeniatus*. (After *Glücksohn.*) × 15.

is straight, and still earlier it shows no subdivision but is uniform throughout its length.

Tracing structure.

5. The *complexity* within the organs decreases quite noticeably. Contributions from different sources which in the later course of events will join to form composite organs are still neatly separated at an earlier stage. Tracing the hypophysis, for instance, which is of composite origin, we come to a stage where the two tributaries, one coming from the central nervous system, the other from the ectoderm, become

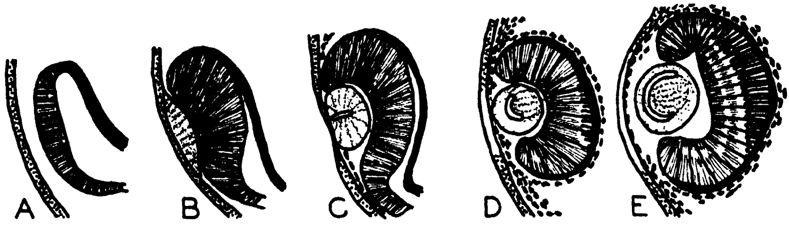

FIG. 11. Five stages in the development of the amphibian eye. (Partly modified after *Rabl.*)

A, Eye vesicle.
B, Invagination of cup; formation of lens placode from skin in contact with optic layer of cup.
C, Eye cup receives lens vesicle which becomes pinched off from skin.
D, Onset of sensory differentiation in eye; beginning differentiation of lens fibers on side facing retina; immigration of mesenchyme into space between eye and skin.
E, Stratification of retina.

dark stippled . . . skin; light stippled . . . lens; hatched . . . retina (optic layer); black . . . tapetum (pigmented layer); heavy dots . . . mesenchyme.

confluent. Furthermore, the content of organs is purer and more homogeneous in earlier stages owing to the fact that strange elements contributed from connective tissue, blood, and the nervous system have not yet mingled with its native elements. Also the organ's own cell types have not yet segregated into distinct groups. The early retina, for instance, does not show the lamination of the later eye, nor does the early eye contain the strange admixtures of lens, cornea, blood vessels and mesenchyme (Fig. 11).

6. The internal *architecture* of an organ becomes simpler, the farther we trace it back. Frequently this is merely a question of numbers insofar as younger organs contain smaller numbers of their constituent elementary units. There are fewer lobes or acini in a young gland; there are fewer plates on a young gill; there are fewer villi in the earlier gut.

7. If we trace back the relations among various organs, we find that their *spatial relationships* have sometimes shifted considerably during development. A striking example is the descent of the testicles into the scrotal sac from a position much higher up in the body cavity. Muscles migrate over considerable distances, and many other organs undergo similar, though less extensive, displacements.

The elements of organ development.

Inasmuch as what we have said here holds more or less for all organs, one thing becomes clear: *the further back we go in the embryonic history, the more do different organs resemble each other,* simply because of the greater primitiveness of their shape, outline, and inner organization. Whether this greater resemblance means greater similarity of character, cannot be decided from pure observation and comparison; analytical study will be accorded to this question on a later occasion.

If we follow an organ back to its earliest identifiable trace, we generally find it as nothing more distinctive than a local accumulation of cells or a patch of cells of slightly different aspect in the midst of other cells. This earliest discernible indication of an organ is called *primordium,* sometimes also *rudiment* or *anlage.* Tracing it further backward, its outline and distinction fade more and more; still earlier, we observe nothing that would suggest the later prominence of the location. Merely in a descriptive sense, extrapolating from what we know about the later events, we may call those locations *prospective organ districts;* this expresses simply that in these places a limb, or an eye, or a lens, or an ear, or a tail will later arise, without implying that those very early stages contain any hint as to the future direction into which their developmental course will turn.

The series of changes which we have here discussed, culminating in the establishment of complex, structurally perfect, and fully functional organs, is called *organogenesis.* The morphological transformations accompanying it are called *morphogenesis,* and the internal modifications affecting the tissues are called *histogenesis.*

Organogenesis.

Organogenesis is only partly a matter of the transformations of individual cells; to a large extent it concerns happenings in which a tissue is involved as a whole. This is particularly true on the morphogenetic side where the same tissues may appear in strikingly different arrangements and produce organs of distinctly different form. Compare the tissue contents of a limb and a tail in an amphibian; both contain epidermis, glandular cells, pigment cells, connective tissue, muscle, tendons, cartilage, bone, joints, blood vessels, nerves. Or compare the antennae and claws of a crayfish; both contain chitin, hypodermis, muscles, nerves, blood. Yet, how different they are in form and structure. The same materials are cast in different shapes as a result of initial differences in the arrangement, grouping, and growth patterns of the respective cellular masses. Such comparisons are useful in making clear the fact that organogenesis cannot be reduced entirely into the sum of individual cell activities, and that it is often of greater import from a morphogenetic standpoint what happens to the cells than what they do individually.

Morphogenesis.

Tracing back *morphogenesis* is tracing back a work of sculpture to its inception from a block of protoplasm. This block is either spherical if the whole egg takes part in development, or is a disc of active substance resting on top of an inactive substratum of yolk in those forms in which only part of the egg goes into the formation of the embryo. This primordial mass is fairly simply outlined, uniform, and continuous in its interior; only gradually does it assume in growing complication the typical outlines and the internal subdivision and organization of, first, the embryo, and, later, in fluent transition, the young and finally the adult body. While growth increases the mass, morphogenesis shapes it.

Morphogenetic movements.

The term "morphogenesis" refers to a multiplicity of happenings; these are partly results of differential growth, partly of organogenesis. A third contributor, however, which is particularly prominent in early stages should not be overlooked: the rearrangement of germinal areas by *movements* before the onset of growth and organogenesis. Through these movements the cellulated materials composing the blastula are shifted relative to one another, bringing each sector into a position where it will be used in the ensuing processes of morphogenesis. Essentially the shifts subdivide the germ into three more or less distinct portions, known as the *germ layers.* The prospective *ectoderm* is left on the outside; the prospective *entoderm* moves into the interior of the germ, in accordance with its later function to produce the digestive system; and the prospective *mesoderm* moves into the spaces between ectoderm and entoderm where its derivatives — muscles, skeleton, blood, connective tissues, uro-genital apparatus — will be needed. In this way the primitive outline and the fundamental plan of the embryo are laid down in very early stages. The phase during which these first formative movements occur has been called *gastrulation.*[1]

In starting morphogenesis with the transport of the major building implements of the body into the places of their future disposal, the organism follows sound engineering practice; for, the first step in a construction job is the carting of the assorted materials to the proper sites. In the arrangement of the germinal materials which results from the gastrulation displacements, one recognizes for the first time a beginning definiteness of form, indicating faintly but unmistakably the future basic plan of the individual. The main axis, dorsal and ventral sides, the median plane and the major symmetry of the body become distinguishable. Through all the later distortions, transformations, and perfections, this basic pattern shines through; all subsequent inner elaboration of the body, involving budding, growth, and shifts of localized organs, remains within this original framework of organization. All later formations appear like ripples superimposed upon a basic wave, the spatial pattern established during gastrulation.

After retracing the general embryonic history of shape and organ formation, let us now focus on the fate of the individual constituent cells, and examine the changes they suffer during embryogenesis. We shall take up the different points in the order in which they appeared in the above listing (p. 57 f.).

The Development of Cell Characters

1. *Shape.* The further we go back, the less definite becomes the shape of the cell, until we come to a primordial stage in which the cell type can no longer be identified by its shape. Nerve cells lack their distinctive processes, sen-

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[1] Although the term "gastrulation" refers primarily to the inward movement of the *entoderm,* in many forms the segregation of the *mesoderm* accompanies this process, so that a sharp distinction between a two-layered and a three-layered phase of the germ is not generally warranted.

sory cells of the eye have no rods or cones, muscle cells are still plump instead of spindle-shaped. The simplest stage of a cell at which its future fate can be told by some discriminating character is usually indicated by the suffix "*-blast.*" Thus we speak of "neuroblasts" as cells which, although still far from having the aspects of true nerve cells, disclose by some index their destination to become nerve cells. We speak of "myoblasts," "osteoblasts," and "hemocytoblasts," as the forerunners of muscle cells, bone cells, and blood cells, respectively. If we follow them back still further, all indications of their future fate are lost. A cell at that stage may be flatter or higher, depending on whether it is stretched or crowded; it may possess cilia in the transitory service of moving the germ about, or it may secrete some liquid or jelly, but all these are temporary functions unrelated to the eventual fate. There is a stage in which only two cell shapes exist: *epithelial* and *mesenchymal* cells; the former lie in the surface, the latter in the interior of the germ. Antecedent to this stage there is only the epithelium of the germ layers, and ultimately, during the stages of segmentation, all cells are *blastomeres* — uniform cells joined by cohesion along their surfaces of contact. In some forms (e.g., insects, birds), the earlier segmentation stages do not even consist of individualized cells at all; the whole germ is a continuous protoplasmatic mass in which cell nuclei lie embedded. So, not only the shape but also the individuality of a cell can have an ontogenetic history.

2. *Size.* The size of the cells becomes the more uniform, the earlier the stages one examines. In fact, the appearance of the most characteristic and conspicuous size differences is a very late event in development. If individual cells grow to markedly larger sizes than the cells of their surroundings (e.g., certain nerve cells, or the sex cells in the gonads), this excessive growth is practically the last thing that happens to them after all the other developmental modifications have run their course.

3. *Functional equipment.* As we have said above, the earlier in its history we examine an organ, the less physiological function does it exhibit; this lack of functional capacity is explained in part by the inadequacy of the functional equipment of the cells. Thus, a myoblast lacks the elaborate contractile apparatus which its derivative, the muscle fiber, possesses; young visual cells lack the typical elongations endowed with those chemically sensitive substances which in the later eye mediate the response to light; and in the forerunners of specific secretory cells the chemical laboratory to produce the specific secretion is obviously not yet ready. Even very young cells may exert some physiological function, such as ciliary beat, phagocytosis, or pigment formation; but these are at any rate far less specialized than the later typical functions.

4. *Reactive properties.* This is a point which is difficult to trace back since by its very nature it does not reveal itself unprovoked. The development of the reactive powers of cells would have to be studied by recording the response of cells to certain controllable stimuli applied at regular intervals during the whole course of development. Our information concerning this subject is extremely poor. We know that many cells possess some reactive capacity of a specific kind long before that capacity becomes biologically important. We know that the secondary sex characters of a rooster, such as comb growth and crowing, which normally appear at the time of sexual maturity, can be evoked very precociously in the newly hatched chick by the administration of the appropriate hormone.[1] One must conclude, therefore, that the cells are ready to react to the hormone while they are still in an infantile condition. However, there is hardly any doubt that if one went still farther back, one would reach a stage at which the cells would be found refractory. Muscle cells of the amphibian iris which contract in response to light in the adult, fail to react in the larva.

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[1] *Domm and Van Dyke*, 1932.

The development of the capacity of cells to react to specifically embryogenetic stimuli will be dealt with on a later occasion.

5. *Motility.* Speaking in general terms, the capacity to move is certainly greater in early than in late stages. Cells which we find firmly lodged inside the bone or cartilage or nervous system of the adult have performed quite extensive migrations in their earlier days.

6. *Mitotic activity.* Although there are exceptions to the rule, in general *cells at the peak of physiological function do not divide,* and, conversely, *dividing cells do not exhibit physiological function.*[1] It is, therefore, not surprising to find that in the course of development, mitotic activity decreases as physiological activities become more prominent. As will be outlined later, a similar inverse relationship exists between cell proliferation and cell differentiation. If we follow a developed cell backward, we see its outlines and structure become less characteristic until we come to a point at which we find it just emerging from a mitotic division as a daughter cell. In many cell types the mother cell shows little specialization. Frequently only one daughter cell goes on to take the course which we have just retraced, while the other daughter cell remains in the more primitive condition of the mother cell, to become a mother cell itself in due time. Thus, mitotic capacity is always retained only by a certain proportion of cells; the younger a developmental stage we consider, the larger is this proportion.

The Development of Cell Communities

1. *Cell numbers.* In most organs the total number of cells, including specific, unspecific, and reserve cells, becomes smaller and smaller the further we go back toward the origin of the organ. Similarly, the total number of cells composing the organism is the smaller the further back we go. The increase in cell numbers does not, however, express

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[1] *Peter,* 1930.

the correct amount of new cell production during development, because at all times there is a certain amount of destruction of cells going on concurrently; the recorded increase in numbers presents merely the balance sheet of the excess of production over destruction.

2. *Cell density.* In many tissues (nervous, connective t.) the packing of cells is closer in earlier stages. This is largely due to the fact that the amount of intercellular matrix produced by the cells increases roughly in proportion to age.

3. *Cell pattern.* The further we go back, the less definitely oriented and the less typically placed do we find the individual cells. Their arrangement is more random, more variable, and lacks the rigid, well-defined character of the formed body. Far enough back we note virtually complete absence of definite cellular arrangements suggestive of the future typical tissue texture except that a large proportion of the cells hangs together in epithelial sheets.

4. *Cell matrix.* Matrix is present in the early stages — indeed as soon as the germ has passed the stage of loosely adhering blastomeres and has acquired some degree of consistency. But these matrices are inconspicuous, fairly homogeneous, and show few of the later typical differentiations such as fibers or deposits of calcareous or collagenous substances.[1]

General Character of Development

In the foregoing discussion only a few representative changes occurring during the development of each cell have been considered. They are sufficient, however, to bring certain general features of development into clear relief. These features are the following.

The general trend of development.

The further we go back in development, the more indefinite and generalized are morphology and function of a cell, the vaguer is its shape, the simpler its structure, the less stable its relation to other cells. Development, as it affects individual cells, implies, therefore, the appearance of new characters which make a cell become different in two respects: first, different *from its own former self*, and, second, different *from other cells* to which it originally bore a close resemblance. These changes relating to the cell and its formed products, constitute the third fundamental phenomenon of development — *growth* being the first and *morphogenesis* the second. We may call it *histogenesis*, or *differentiation* in the strict sense.

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[1] The question of the origin of this matrix in the embryo has not yet been brought to a final solution (cf. *Snessarew*, 1932; *Studnička*, 1934).

The gross lines of the development of an organism become evident when one contrasts the conditions in which its cells are found at the beginning and at the end of their careers.

TABLE 4

Trend from earlier stages toward later stages

From	Toward
uniformity	diversity
variability	stability
vagueness	definiteness
ubiquitous occurrence	local restriction
plasticity	rigidity
irregularity	regularity
generality	specialization
mobility	immobility
simplicity	complexity

This table expresses in just so many words the general trend of development as we have come to see it. This trend is fundamental to *all* animal development, however much variation in detail may separate the different forms. If development has been identified with progressive change, we now recognize the general direction which the progress takes. Some may regard this as an important result; others will call it an obvious generality. As a matter of fact, embryology has long suffered from the delusion that the basic problems of development could be really solved by mere reference to generalities of this kind. Every attempt made thus far to "explain development" on the basis of a *single* principle bears this stigma. Of course, it is comparatively easy to conceive of models and analogies that would adequately reproduce any one or several of the general trends listed above, but no critical mind would accept them as "explanations of development." For the most part, "explanations" of this sort cannot survive the first rigid test on a concrete phenomenon of development. They may have philosophical merits; but their scientific value remains to be demonstrated, if we accept the attained degree of control and predictability of natural phenomena as the criterion of scientific proficiency.

In order to avoid the ever imminent danger of falling into this same course along which lie all the real impediments to scientific progress, such as oversimplification, sham solutions, unverifiable assertions, etc., it is advisable not to think of development solely in those abstract terms in which it has been represented in the above table, but rather to stay as close as possible to the ground and to the specific phenomena observed. "Differentiation" should not remain a vague and abstract notion; concrete examples of what actually happens to a cell in differentiation should be present in our minds whenever we refer to it. The same haziness which used to attach to the term "development" still vitiates many a discussion of the problem of "differentiation." The obvious remedy is to be explicit. This is the purpose of the following account of the actual happenings during cell differentiation.

A SPECIFIC ACCOUNT OF DIFFERENTIATION

Cell Division and Cleavage

The birth date of the cell.

A cell dates its existence as an individual from the time of its last division. It was then that a mother cell gave up its identity by splitting into two daughter cells. Since this happens over and over again in the course of development, it follows that most of the cells with which we deal in the adult organism are much younger than the germ as a whole. The cells as individuals are born anew with each successive division. The situation is different, however, if we consider the cell not as an individual unit, but as a member of the continuous series of cell generations descending from the egg. For, inasmuch as every new cell individual consists of part of the protoplasm of the mother cell, just as the latter is a continuation of the protoplasm of its ancestors' cells, there is protoplasmic continuity from each cell back to some protoplasmic unit, however infinitesimal in size, of the undivided egg. Thus the question arises as to whether cellular differentiation is confined to the life span of the individual cell, or transcends it so that a dividing cell would pass on its differentiations directly to its progeny.

Differentiation in cell strains.

Is differentiation a product of the cell individual or of the cell generation? The answer is different for different manifestations of differentiation; it varies depending on whether we have in mind microscopic cell structure or the general character of the cell. Much of the former (membranes, fibrils, inner framework, canals, etc.) must be re-formed in each cell individual. For, we know that the act of mitosis causes such a thorough stirring up of the cell content that many of the internal features which the mother cell has managed to produce during its previous interkinetic phase are wiped out, or at least upset. Consequently, a daughter cell emerging from a division cannot continue with the elaboration of its physical equipment from exactly the point where the mother cell has left off before entering into the mitotic act. But, while the mitotic upset has far-reaching consequences for the *physical* structures of the mother cell, it does not interfere very seriously with the *chemical* distinctions a cell might have acquired.[1] Mitosis is a kind of earthquake for the cell; the most solid arrangements suffer most, while building materials, plans, and some of the mobile equipment can be salvaged into the following period of reconstruction. That is to say, the actual mechanical structures (e.g., tonofibrils) are abolished by mitotic division, while chemical equipment and capacities that go with it can be carried over into the progeny. Certain phases of differentiation must, therefore, be considered as a matter not of the individual cell but of the whole cell strain. Now, inasmuch as all cell strains descend from the same egg cell, one ought to expect that if they all started to differentiate at the same time and proceeded at the same rate, the cells of any given stage of the organism would all be differentiated to the same degree; in other words, be of identical age of differentiation. But this is by no means true.

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[1] For a brief discussion of this problem, see *Berrill and Huskins*, 1936, and *Dawson*, 1937.

As we have already mentioned, the organism harbors cells of very unequal degrees of differentiation and maturity (p. 56). Only a certain proportion of cells is in a state of maximum efflorescence, as it were, while others lag behind, comparable to buds, and still others — reserve cells — are arrested at a relatively undifferentiated level. Just as one occasionally sees buds, blossoms, and fruits on the same citrus tree — the whole sequence of stages exhibited simultaneously —, so samples of the major steps of cell differentiation can be found coëxisting in the same individual. Obviously, therefore, some of the cell strains must have started to differentiate later than others, and if we depict the whole progeny of the egg cell in the form of a tree, we realize that only in certain branches has differentiation taken a steady and uninterrupted course while in others progress has been suspended for varying lengths of time. It is this delay which is responsible for the increasing discrepancy in the degree of differentiation observed among cells which otherwise are contemporaries. Some cells are merely delayed and later catch up with their precocious neighbors, while others may be permanently arrested at a stage of differentiation inferior to that which we know lies within their capacity to attain. The reason why cells of identical origin should diverge more and more as development goes on will not concern us until later. For the present, we simply record it as one of the basic facts of differentiation. For the sake of illustration, let us trace the genealogy of one or two particular cell strains as they spring from successive cell divisions in a developing embryo.

Cleavage.

The egg divides by a number of more or less synchronous cell divisions into an increasingly larger number of blastomeres. This process is known as *segmentation* or *cleavage.* Only that part of the egg which consists of true oöplasm is broken up into cells, while those portions which consist mainly of yolk remain either unsegmented or cleave with considerable delay. Hence the completeness with which an egg is broken up into individual cells by cleavage is in inverse proportion to the amount of yolk present (compare Figs. 35 and 36).

Reduction of cell size during cleavage.

Cleavage is in many respects quite different from cell division in post-segmentation stages; the most notable difference is that in the former process successive divisions are not separated by growth phases. In later embryonic development, as well as in the adult organism, a cell must grow, usually to approximately twice its original volume, before it is again ready to divide; successive steps of division are, therefore, separated by such intervals as are necessary to allow the daughter cells to regain size. No similar growth phase, however, intervenes between the steps of division during cleavage. The cleavage divisions follow each other in rapid succession and the size of the cells so produced decreases steadily as their number increases. During the earlier stages, then, the mass of the egg as a whole remains fairly constant, save for minor fluctuations caused by the intake of substances from the outside medium and some secretion into the inside of the germ to fill the blastocœle. *Cleavage, therefore, is not associated with any appreciable increase in the mass of living material available for development;* it merely involves the *breaking up of the living substance of the egg into a great number of individual cells.*

Reduction of nuclear size during cleavage.

Essentially this is also true of the egg nucleus (germinal vesicle). The nucleus of the activated egg (fertilized or parthenogenetic) becomes subdivided into numerous nuclei of much smaller size, one to each blastomere. In eggs with total cleavage the nucleus is comparatively enormous and could easily accommodate a large number of whole cells taken from a later stage. Out of each cleavage step, two daughter nuclei emerge each of which is just a little larger than half the volume of the mother nucleus. The fact that the volumes of the two daughter nuclei add up to slightly more than the volume of the mother nucleus while at the same time the cytoplasm is more or less exactly halved by each fissure indicates that there is a certain amount of nuclear growth during cleavage at the expense of the surrounding cytoplasm. The following figures illustrate the relationship as found in the development of the sea-urchin. Comparing a cell of the young larva with a single blastomere of the two-cell stage, one finds that the former is about 2500 times smaller than the latter. That is, the cellular volume has decreased during the embryonic period at a ratio of 1:2500. At the same time the ratio of the nuclear volumes of the same cells has only decreased at the rate of 1:700.[1] Hence the ratio between nuclear volume and total cell volume has been raised by about three and one half times in the course of development.

Early differentiations.

At the end of cleavage, the cells present slight inequalities; these do not seem to have developed so much during cleavage as to have been pre-existing in the egg; that is, cells arising from different parts of the egg receive slightly different allotments of the egg content. The visible differences among the cells at this stage are largely confined to differences of size, height, and content of granules, pigment, and yolk. More characteristic differences develop during the ensuing stage of gastrulation; cells belonging to the different germ layers can then be distinguished roughly, though none of their later specialized characters have as yet appeared. Cell size has in the meantime approximated its fixed minimum, and mitotic activity is temporarily at a low mark. Henceforth, a cell will divide only after it has grown; this growth is made possible in lower forms by the inception of larval life with the intake of food, and in higher forms by the beginning assimilation of stored yolk into living protoplasm.

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[1] *Rh. Erdmann,* 1909.

The Fate of Cell Strains

Early changes of cell shape.

Let us now select two particular cells of a vertebrate germ at this stage and follow their and their descendants' adventures (Fig. 12). The first cell we choose is one belonging to that part of the ectoderm in which the nervous system is to arise (compare p. 494). When we first study it, this cell is a simple epithelial cell, and nothing reveals its future fate. It lies amidst scores of other cells and looks exactly like the rest. It moves with the others during gastrulation toward what is to become the dorsal side of the germ. There it begins to transform: it becomes higher, larger, and its nucleus assumes a characteristic position (Fig. 111, p. 502). Thus, it becomes distinguishable from ectoderm cells in other regions of the germ. Part of this change may be a passive result of crowding and of other changes in spatial relations forced upon the cell, but primarily we are dealing with an intrinsic change in cell character. As we shall see below (p. 499), these cells have developed a greater avidity for water, for they swell much more than do other ectoderm cells; this indicates that they have suffered very thorough changes of chemical and physico-chemical constitution (p. 32). In this condition the cell is called a medullary cell (see p. 512); let us designate it as *A*. It is characterized as much by the loss of certain primordial aspects and faculties which it had formerly shared with others as by the appearance of certain new characters distinguishing it from other cells that have failed to move in the same direction. There are all sorts of new performances connected with the new condition, such as, for instance, secretion (see p. 500), but we shall not go into further details. The main thing to keep in mind is that the cell has undergone this transformation without dividing.

Division is resumed.

This changes now; while other cells, late-comers, undergo a similar transformation, our cell stops for a while the work of inner perfection and prepares to divide. The division abolishes some of the previously attained characters such as shape, orientation, nuclear position, etc. (cf. Fig. 111), but these are quickly resumed after the daughter cells have separated. Thus the daughter cells turn out to be of a stage of differentiation corresponding to that of their mother cell: stage *A*. Apparently, many advanced characters of the mother cell were handed down directly to the daughter cells. From the mother cell a daughter cell inherits that part of the finished equipment which is not destroyed by the act of mitosis, and, for the rest, the capacity to build up quickly a similar equipment of its own. A mother cell which has swollen because of the greater imbibition of its protoplasm passes on the greater swelling capacity as well as the higher water content of its substance.

The cell strains branch.

Cell *A* divides into two cells; these again in two; and so forth. They are all medullary cells. Without changing noticeably in their constitution, they continue to divide for a certain period and produce a numerous progeny of similar looking cells. In the meantime, differential growth and the folding and closing of the medullary system (see p. 497) have carried our *A* group into a localized site. Although the stereotypism with which these movements and growth differentials recur in every individual case allows us to predict the general area of the later germ into which the derivatives of a given cell will come to lie, the exact spot in which any one of our *A* cells will be found in the formed neural tube is accidental. It can safely be stated, at least for higher animals with very large numbers of cells, that there are no two individuals in which the progeny of any given cell is spatially

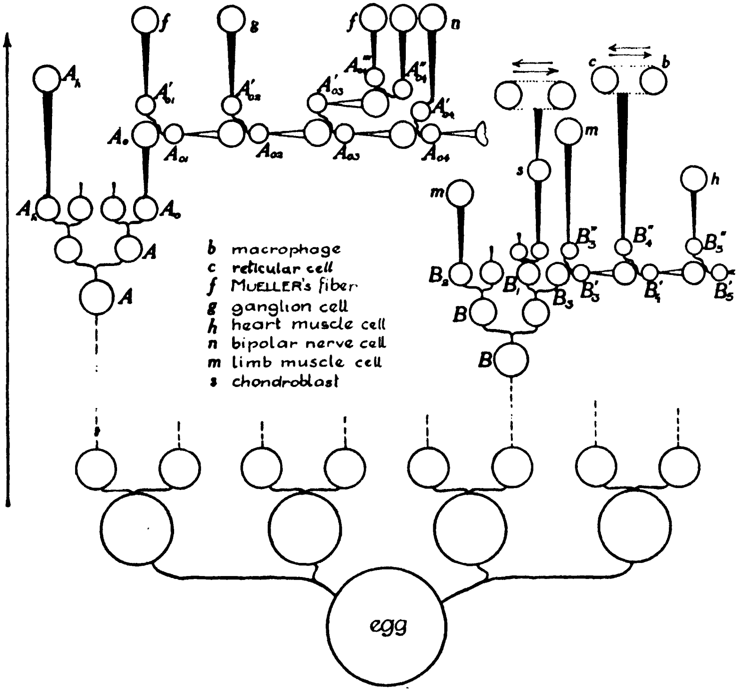

FIG. 12. Diagram illustrating the differentiation of various cell strains in a germ. Vertical direction (bottom to top; see arrow on left) marks progress of differentiation. Horizontal direction marks changes not attended by cellular differentiation (proliferation; modulation). Black wedges indicate cell growth with attending differentiation; white wedges, cell growth without differentiation. For further explanation, see text.

distributed in exactly the same way. Thus the individual members of our A strain end up in a variety of situations, subjected to slightly different conditions. Their fates, henceforth, separate like those of the members of a large family scattered all over the world.

Following two branches.

Let us abandon most of these A-descendants and merely select two for further tracing; we shall call them A_h and A_o. The former may lie in the floor of what is to become the mid-brain, while the latter may be situated a little more laterally in the region where the eye vesicles begin to bulge out. Let us assume further that the former has been pushed toward the outer wall of the brain while the latter has remained in contact with the inner (ventricular) brain surface. A_h, now identifiable as a so-called *spongioblast*, shows marked morphological changes. It forms protoplasmic processes which soon assume a wiry appearance. Along with this development many other aspects of the cell have changed. Its interior looks different; its nucleus is different; its reactions to histological stains have become elective. From now on it has a definite character by which it can be identified. It is now called a *glia cell.* Its differentiation has reached a peak, and one branch of the tree of A cells has thus come to a dead end. For an abbreviated genealogy of medullary cells, see Figure 13.

A_o has in the meantime divided again into A_{o1} and A'_{o1}. Daughter cell A_{o1} remains in the position of the mother cell, that is, in contact with the inner surface of the brain, while A'_{o1} leaves this surface and moves centrifugally into the brain wall which in this district, according to our assumption, forms part of the eye vesicle. After a certain interval during which it has grown, A_{o1} divides again in a fashion similar to the division of A_o; that is, it gives rise to one superficial (A_{o2}) and one deeper cell (A'_{o2}). The cells A'_{o1} and A'_{o2} which have moved away from the free ventricular surface cease to divide; instead they continue with their

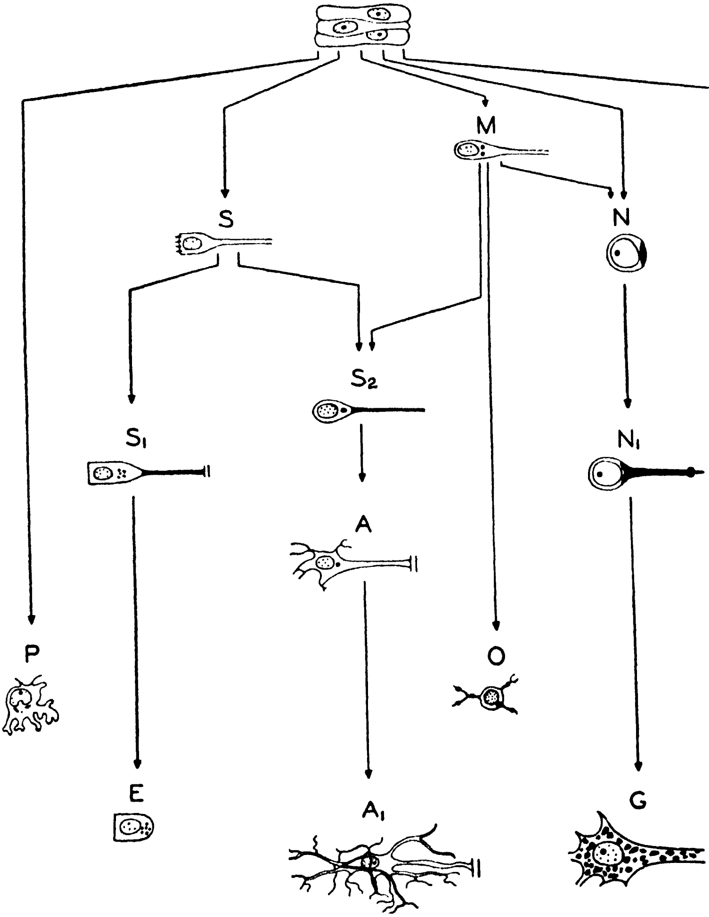

FIG. 13. The medullary cell strain. (Simplified after *Bailey.*) The diagram represents the main cell forms descendant from the cells of the neural epithelium.

P, Pineal parenchyma; S, Primitive spongioblast; S_1, Ependymal spongioblast; E, Ependyma; S_2, Unipolar spongioblast; A, Astroblast; A_1, Astrocyte; M, Medulloblast; O, Oligodendroglia; N, Primitive neuroblast; N_1, Unipolar neuroblast; G, Neuron; T, Chorioidal epithelium.

histological differentiation. The proliferating activity is carried on by that part of the strain which has remained in contact with the surface. In these cells differentiation is definitely delayed. In contrast to the progressive changes occurring in A'_{o1}, A'_{o2}, etc., cells A_o, A_{o1}, A_{o2}, etc. remain thus in what appears to be a relatively undifferentiated condition.

Reproduction vs. differentiation of cells.

This splitting of descendants of common cells into one line that remains *reproductive,* and numerous side lines sprouting off from the former and entering into *differentiation,* is one of the basic features of advanced stages of development. In fact, it is only by virtue of this distribution of tasks to different cell groups that growth and differentiation of an organ can proceed concurrently. Cellular differentiation and cellular multiplication are two processes which, if not strictly mutually exclusive, are nevertheless markedly antagonistic in their tendencies. Therefore, in general, a cell body does not divide and differentiate at the same time. This point, briefly alluded to before (p. 72), deserves some additional remarks.

We have said that mitotic and functional activity of a cell are not absolutely compatible; neither are cell division and cell differentiation. Apparently a cell whose content is continually stirred up in the service of division lacks the necessary tranquillity to build up definite mechanical structures. Just as the stirring of boiling milk prevents the formation of skin on the surface, and the stirring of muddy waters prevents the silt from settling, so does the repeated mobilization of the cell protoplasm by mitotic activity prevent the condensation, orderly precipitation, and regular segregation of intracellular substances into differentiated structures. On the other hand, if a cell has had the necessary rest and has transformed a major part of its protoplasm into specialized intracellular apparatus, the attendant consolidation of its substance seems to make it increasingly difficult for the protoplasm to return to such plastic condition as is required for the act of mitosis. Consequently, we realize that conditions which favor either cellular division or cellular differentiation are at the same time unfavorable to the other.[1] This statement holds primarily with regard to morphological structures, whereas a steady change in the chemical and physico-chemical properties of the cellular content can continue despite periodical mitotic agitations (see above, p. 76).

The germinal layers.

In general, there is a certain spatial separation between the cells of the mitotically active line and their differentiating offspring. In our present example, for instance, mitotic divisions are confined to the inner (ventricular) surface of the brain and eye vesicle. In the epidermis of the skin proliferation is restricted to the basal layer, in the lens of the eye to a group of cells arranged along the equator, and a similar localization of the proliferating source is observed in most growing organs. The proliferating portions of an organ or tissue are generally designated as *germinal.*

Divergence of differentiations.

The cells budded off from the germinal layer[2] and proceeding to differentiate can develop in a variety of directions, essentially in accordance with the situations into which they have drifted. In the case of our example, the cell A'_{o1} (see Fig. 12) has become transformed gradually into an elongate cell of considerable consistency spanning the thickness of the optic layer and forming a reinforcing fiber in the retina (Müller's fiber). Another cell, A'_{o2}, may have moved across the retina; upon reaching the opposite surface it settles down, produces a long nerve fiber and thus assumes the character of a ganglion cell of the ganglionic layer. A third cell from the same source, A'_{o4}, may have settled somewhere in the middle layers of the retina producing a bipolar nerve cell. Still another cell, A'''_{o4}, may again become a supporting cell like A'_{o1}. It is needless to enlarge upon these examples any further. Eventually, when the proliferating activity in the germinal layer subsides, differentiation takes hold of this portion, too, and gives rise to the visual cells, rods and cones (see Fig. 11).

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[1] The existence of some sort of antagonism between cell proliferation and cell differentiation was inferred from the periodicity of the embryonic growth of the chick reported on page 132 (*Schmalhausen*, 1926). The point has been brought out, however, far more stringently by tissue culture experiments in which the deliberate enhancing or depressing of growth had just the opposite effects on differentiation (*Strangeways*, 1924; *Fischer and Parker*, 1929).

[2] Not to be confounded with "germ layers"; see page 68.

Several important features of differentiation can be immediately deduced from this schematic picture.

Age differentials.

First, we understand how an organ happens to present such a heterogeneous mixture of cells in various degrees and stages of differentiation during its development (p. 56). The reason is that the progress of differentiation is, if not absolutely suspended, at least appreciably delayed and retarded for the cells of the germinal line. Only after cells have left the germinal layer do they continue to differentiate at a normal rate, and since they spring from the mitotic line at different times, it is clear that we must find all degrees of differentiation sampled in the organ at the same time. Compare, for instance, cell A'''_{o4} with cell A'_{o1}, both of which give rise to Müller's fibers. A'''_{o4} is three generations removed from A'_{o1}; it is the "great-grandchild" of A'_{o1}'s "sister." Consequently we cannot expect them to be in the same stage of differentiation and maturity.

The ratio between dividing and differentiating cells.

Secondly, if a cell of the germinal line divides, there are three possibilities regarding the fate of the daughter cells. (a) Both may continue to divide, causing bifurcation of the germinal line (compare the sister cells A_{o3} and A'_{o3}). (b) Both may be eliminated from the germinal epithelium and proceed to differentiate (see A''_{o4} and A'''_{o4}). (c) One cell may continue to divide while the other may be shifted into the differentiating group. Case (b) means the end for that particular germinal line. Growth by production of new cells can continue only so long as at least part of the germinal lines are going.

Thirdly, if we assume that the choice among these three possibilities is a matter of mere chance, their probable incidence would be as follows: (a) and (b) would have equal chances to occur, for both daughter cells are as likely to quit the germinal layer as they are to stay there. In effect, however, one occurrence (a) plus one occurrence (b) would amount to the same as two occurrences (c). Consequently the average fate of a pair of daughter cells would be for one to remain in the mitotic line and for the other to join the differentiating group. This would have an important bearing on total growth. For, if each successive step of division eliminates one cell as a potential producer of new cells, the number of actively proliferating cells remains more or less constant while the total mass of cells is steadily augmented. Therefore, the *relative size* of the proliferating source in proportion to the total mass of organ already produced decreases more and more; this fact, then, accounts readily for the observed decline of relative growth rates during development. It explains a situation which we have simply accepted as a fact in our discussion of growth (p. 17). A simple example may serve to illustrate this.

Decline of growth rates owing to differentiation.

Let us consider a particular area containing ten cells in mitosis and twenty cells in differentiation, making a total of thirty cells. Assuming that the mitosis of the ten active cells yields ten cells which differentiate and ten others that remain capable of proliferating, we obtain an increase of the total mass from thirty cells to forty, i.e., a thirty-three percent increase. In the next step of division another ten differentiating cells will be added to the mass of the body with an equal number remaining ready for further division, and so forth. The original mass of thirty cells, therefore, jumps at equal intervals to forty, then fifty, then sixty, then seventy, etc., which, expressed in percentage increase, means relative additions of thirty-three, twenty-five, twenty, seventeen, and fourteen percent, respectively. The gradual decline of the *relative growth* rate becomes quite obvious in these figures. Under natural conditions the situation is not precisely as simple as here assumed because in reality the distribution between proliferating and differentiating cells is not merely a result of chance. As a matter of fact, the germinal lines may at times split repeatedly as in (a) and thus throw the balance over in favor of the multiplying cells, whereas at other times of development they may become exhausted much faster than should be expected according to probability. Then the growth of the organ stops entirely. This does not alter, however, the basic conclusion that the decline of growth rates with the progress of development is primarily due to the gradual elimination from mitotic activity of the differentiating cells. In some cases all available cells are disposed of by differentiation, exhausting the reservoir of proliferation. This is true, for instance, in the development of the central nervous system of man, in which the production of new nerve cells is definitively ended shortly after birth. In most organs, however, there is always a remnant of the germinative layer left which, if not in permanent proliferation, can be made to proliferate by suitable stimuli and from which regenerative processes may originate.

Further examples of divergent differentiation.

Let us now turn to a different strain of cells selected from the middle germ layer, the mesoderm of a late gastrula. We choose a cell which lies close to the notochord in the mesial portion of the *myotome* (*B*, Fig. 12). The cell keeps dividing; one of its derivatives leaves the rest and moves amidst other scattering cells toward the notochord whose surrounding cell layer it joins. We abandon this cell right here, merely indicating that it is later to transform into a *chondroblast* (B_1) and, after some additional divisions, into cartilage cells. We return to the cells that have aligned themselves in the myotome (B_2).

These become very elongate myoblasts (m); they produce contractile fibrils in their interior. The formation of these intracellular fibrils represents exactly that type of morphological differentiation whose existence would be threatened by cellular division according to what we have stated above (p. 76). In the present object, however, a certain modus vivendi has been established between the two opposed processes of differentiation and proliferation. Part of the cell differentiates, while another part of the cell is in the service of proliferation without disrupting the differentiating portion. This implies, of course, that the act of division remains incomplete, and the cell, instead of dividing into daughter cells, confines itself to having its nuclei divide into daughter nuclei which remain embedded in a common syncytial cytoplasm. In other words, the nuclei propagate while the cytoplasm increases in mass without dividing and so continues its internal differentiation without interruption.

The same results obtained by different ways.

If we follow another mesodermal strain (B_3) we see the cells divide, move, change their form, assemble, scatter again, but nothing decisive seems to happen. These cells form the so-called *mesenchyme.* They fill the body spaces of the primitive embryo in a capacity of primordial connective tissue and then lie in wait for later needs of differentiation that may arise. Cell B_3 may, for instance, appear in a local thickening of the body wall representing a limb rudiment. Following one line of descendants of B_3, we notice cells (B''_3), which elongate markedly, produce muscular fibrils in their interior and become myoblasts (m) just as in the strain B_2. They form muscle fibers. Neither these, nor the muscle fibers differentiated from the myotome actually contract by the time the fibrils are developed. The reason is not lack of contractility but the absence of a suitable stimulus to elicit contraction; for, when artificially stimulated, they twitch even in those early stages. The differentiation of these cells has, therefore, not been restricted to the formation of visible structures, but has produced the whole complicated chemical and physico-chemical mechanism underlying irritability and contractility. It is instructive to note that both strains, B_2 and B_3, have led to the same products — muscle fibers — although in different ways. A muscle fiber which develops from mesenchyme in the limb has never shared in the epithelial arrangement of the myotome, has immigrated into the limb bud as an independent single cell and only there has associated with more of its kind.

Other cells of the B_3 strain continue to divide (B'_3, B'_4, B'_5) giving off side lines of differentiating or otherwise non-prolific cells (B''_4, B''_5). Among these appear more muscle cells, cartilage cells, connective cells, blood cells, and others.

Arrested differentiation.

However, only a certain fraction of the mesodermal cells reach a state of final functional specialization such as that of muscle cell or bone cell or blood cell, while a considerable number remain in a more primitive and indefinite condition. This is also true of the ectodermal and entodermal derivatives, although less extensively. In any case, the presence of a certain amount of cells arrested at a lower level of differentiation is the rule even in the fully developed organism (p. 468).

The end stages of differentiation.

It is by no means easy to arrive at an agreement as to what to consider as the end point of differentiation. It was easy to determine the starting point, which is the egg; but can the terminal point be defined with the same certainty? Apparently not. It is only in exceptional cases that the morphological cell characters are so constant and rigid as to present us with a reliable criterion of the degree of differentiation. After all, a completely stable and quiescent cell is a dead cell. Change is inherent to life, and so long as the living activity lasts, the cell is in a state of flux. Fluctuations may be greater or less, but they are practically always present in a certain measure. The same cell individual may assume the most diverse aspects under varying circumstances. This, of course, makes it practically impossible to define cell *character* unequivocally in terms of cell *appearance.* One of the characteristics a cell acquires in the course of differentiation is its ability to operate. This involves activity, change; it implies that a cell at times will be found in a more resting condition and at other times in a more active condition, or that a cell at one time will be engaged in one type of activity and at another time in a different type of activity. The microscopic aspect of the cell will vary to the extent to which these functional activities have structural counterparts.

Here is the place to return to the distinction made above (p. 11) between physiological and developmental changes. The physiologically active cell at the end of development fluctuates about an average condition without deviating permanently from this state. Or, reversing this statement, we may agree to consider the differentiation of a cell as terminated when it has attained a stable level from which there can be no further progress. This implies neither rigid form nor lack of change; it merely implies that whatever change occurs will be *repetitive* rather than *progressive.*

The plasticity of the differentiated stage.

In this view, the differentiation reached by a given cell is understood to include all the *specific reactions of which the cell is capable.* Thus the differentiated stage of a muscle cell, as discussed above, is characterized by the presence of fibrillae and other visible elements as well as by the capacity of these fibrillae to contract if and when a suitable stimulus impinges upon them.

Another example will make the point clearer: the *chromatophore.* These pigment cells in the skin of many animals are known to expand in darkness by sending out fine pseudopodia in which the colored granules are suspended; in this condition the chromatophore resembles a lichen. In light, however, the pseudopodia are withdrawn and the cell body contracts into a compact clump. Now one might ask, which one of these states should be considered as the "differentiated" stage of the chromatophore — the light-adapted or the dark-adapted one? It is obvious that the whole question is meaningless because the cell can freely shuttle back and forth between the contracted and the expanded state in repeated adaptation to the changing stimulus situation. Consequently, a concise definition of the *differentiated stage of a pigment cell* can be given only in terms of its reactive properties; that is, by defining it as a *cell which, in the face of such and such stimuli, will assume such and such shape, function, etc.* Accordingly, the difference between the differentiated states of a muscle cell and of a pigment cell consists not only in that the former possesses fibrillae and the latter granules, but more pertinently in that the former responds to a different set of stimuli and by a different type of reaction than the latter.

"Modulation" of cell types.

In certain types of cells the latitude of physiological adaptations may be far in excess of the relatively narrow range of variation exemplified by the chromatophore. This holds especially true for cells of the mesodermal kind. For instance, it is an established fact that a spindle-shaped, sedentary, fiber-producing cell of the reticular tissue can react to certain pathological events in the body with very startling transformations. Such a cell can suddenly be mobilized, lose its connections with neighboring cells, become plump, amoeboid, and phagocytic — in other words, assume all the morphological and functional properties which are exhibited by free macrophages. Conversely, under certain other conditions, free macrophages can settle down and transform into fixed tissue cells of the spindle-shaped type. The whole series of gradations between the two extremes is known. Differentiation of this type of cell, therefore, involves, among other things, a capacity of the cell to react to one set of conditions by assuming shape and function of a fixed tissue cell, and to another set of conditions by assuming aspects and functions of a free blood cell. Apparently the same cell can commute back and forth between the two stages, according as the conditions vary.[1] (See the fate of cell B_4 with variable end stages b or c; Fig. 12).

One realizes that the difference between this sort of oscillation of cell state and that observed in pigment cells is only a difference of degree and not of kind. This physiological, strictly nonprogressive fluctuation of a cell in response to its environmental conditions may be called *modulation.* It provides for a certain latitude within which a cell can comply adequately with certain variable functional demands of the developed body. Both character and range of modulation are strictly circumscribed by the specific constitution which the cell has acquired in the process of differentiation. Particularly striking examples of modulation are supplied by the reaction of cells to specific hormones.

Hormone reaction as example of modulation.

A *hormone* is by definition a specific chemical substance produced in one part of the body and exerting specific effects upon distant parts of the same body. Hormones are messengers putting distant organs into communication with each other by the channels of the blood rather than by nervous transmission. A hormone is carried with the blood and, therefore, diffused rather indiscriminately all over the body. If it is capable of inciting specific localized effects in certain parts to the exclusion of others, this must be ascribed to the fact that the affected parts are predisposed to respond to that hormone. We know that many organs in the service of reproductive function respond to the circulation of sex hormones. Consequently, the differentiation of these responsive cells must have included the acquisition of a specific predilection for the sex hormones.

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[1] For further details, see *Bloom,* 1937.

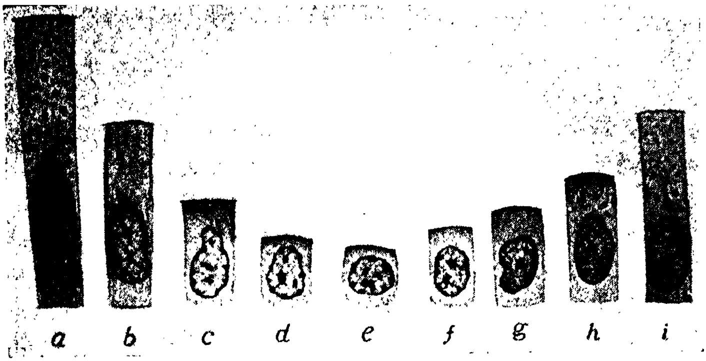

FIG. 14. Effect of sex hormone on the cells of seminal vesicles in the rat. (From *Moore*, *Hughes* and *Gallagher*.)

a, Cell of normal individual in full hormone activity. Note secretion granules in cytoplasm.

c, Cell of hormone-free castrate.

b-d, Intermediate castration stages.

f-i, Return of active state after injection of male sex hormone. Note the reappearance of secretion granules.

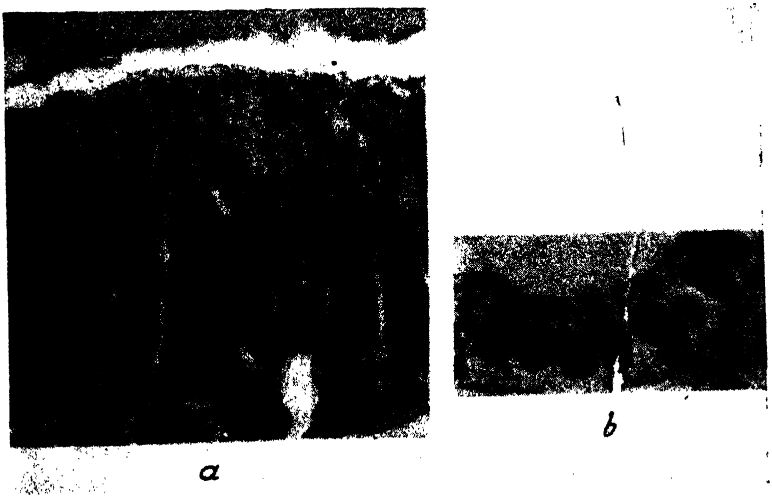

FIG. 15. Effect of sex hormone on the epithelium of the seminal vesicles. (Preparation of *D. Price*.)

a, Seminal vesicle epithelium of animal in active hormone condition (stage *a* of Fig. 14).

b, Same epithelium in castrated animal (stage *e* of Fig. 14).

Both photographs taken at the same magnification. Note the striking difference in size and appearance of the cells and nuclei in *a* and *b*.

In the presence of full hormone supply the cells of the seminal vesicles of a rat present themselves as in Figure 15a. They are elongate cells with large nuclei; numerous secretion granules in the cytoplasm are evidence of an active state of secretion. If the hormone supply is stopped by castration, the cells undergo the marked modifications illustrated in Figure 14, a–e: nucleus and cytoplasm decrease in volume and change in many regards; secretion ceases (Fig. 15b). But as soon as sex hormone supply is restored to the body by injections, the cells revert to their former condition (Fig. 14, f–i), increase in size and resume secretion.[1] At the end we find the cells in the same state (Fig. 14i) as at the beginning (Fig. 14a); they have gone through a full cycle of transformations, with no permanent change, no addition, no loss; hence a *physiological* cycle, not different in kind from the cycle of a pigment cell: contraction — expansion — contraction; or of a connective tissue cell: fixed phase — free phase — fixed phase.

Another example of modulation in response to hormones shows the transformation of a bone cell into a macrophage and a spindle-shaped connective tissue cell of the fibroblast type, and back to the osteogenic (bone-producing) condition of the bone cell under the action of toxic doses of parathyroid hormone.[2]

Some Principles of Differentiation

The principle of progressiveness in differentiation.

The main objective of these examples is to emphasize that the state of cellular differentiation must be conceived of in terms of attending *potentialities* and *activities,* rather than by reference to definite morphological structure. In no case is the microscopic picture which we obtain of a fixed, preserved, dead cell wholly representative of the cell character, because the variety of expressions to which the live cell is amenable under a variety of stimuli fails to show in the picture. To be sure, some cells are so highly specialized that their range of modulation is negligible, and in this case the microscopic picture comes indeed very close to being typical; but in other cases, such as the ones described above, the range of cellular reactivity may be immense, and in that case only the *whole range*, but no single phase cut out from it arbitrarily, is representative of the constitutional characteristics which the cells have acquired through differentiation.

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[1] *Moore, Hughes* and *Gallagher,* 1930.

[2] *McLean* and *Bloom,* 1937.

The physical modifications which a defined chemical compound can undergo, while retaining its chemical identity, are a fitting analogy to modulation; for instance, the transformation of H_2O into vapor, water or ice.

Of course, the criterion by which we are to tell *modulation* from *differentiation* is entirely empirical. It is based on the fact that modulation is a physiological process, *reversible without residue*, allowing a cell to return to exactly the condition in which it was before; while differentiation is *progressive* and involves changes in the cellular *constitution* which amount to either *permanent gains* or *permanent losses.* We are speaking here merely of normal development during which changes of unquestionably unidirectional character are a matter of record. These changes never are reversed. Whether this is because they *cannot* be reversed or simply because the necessity for such reversal never arises is quite another question which cannot be solved but by experimental procedures and which will be dealt with on a later occasion (p. 466). The difference between modulation and differentiation is symbolized in the diagram (Fig. 12), in which a single vertical arrow represents the line of progressive differentiation, while reciprocal horizontal arrows indicate reversible modulation.

The principle of discreteness of differentiations.

There is another significant difference between modulation and differentiation; it lies in what we may call the *discrete-*

ness of differentiations. This term means that differentiation produces a definite number of discrete, distinct, discontinuous and more or less sharply delimited cell types which are not connected by intergradations; whereas in modulation there is a continuous transition of stages leading from one end of the cycle to the other. A nerve cell has a very definite character, and so have a muscle cell, a secretory cell of the intestine, a red blood cell. We know of no intermediate forms between these various types. There is nothing known that would resemble a blended hybrid between a nerve cell and a muscle cell,[1] and an individual cell can only assume the distinct and specific character of either one or the other. Considerable importance attaches to this fact because it contains a clue by which to judge the relative parts played in cellular differentiation by immanent and by extrinsic factors. We shall see later that this touches really upon one of the most fundamental problems of development — does a cell differentiate according to its own devices, or is it molded by influences of its environment? The discreteness of cellular types is strong evidence for the former view. The inner environment of the body exhibits an infinite number of shades; if cell differentiation were determined by it, one ought to observe a practically unlimited variety of cell forms arranged in a graded series. Instead there exists in each species only a limited and comparatively scanty choice of differentiations. Obviously, therefore, cells possess a definite innate vocabulary of differentiations and do not learn their language from the surroundings. This point will be amplified further below to such an extent that there is no need of dwelling on it here.

To summarize, if we symbolize the different courses of cellular differentiation by a number of channels, then all these channels leave from a common center — the undifferentiated stage of the cell. By and by they diverge as the cells enter diverse lines of differentiation. All differentiation moves over these definitely established lines, and random, pathless, straying does not occur. Nor are there cross channels interconnecting the separate lines; once separated, they remain separated. The width of the channels represents the amount of latitude (modulation) left to the cells.

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[1] The so-called Purkinje fibers of the heart which form part of the impulse-conducting mechanism and as such combine functional traits of nerve fibers with morphological features of muscle fibers, nevertheless cannot be regarded as a histological blend between nerve and muscle, but are simply modifications of true muscle fibers.

The relation between differentiation and modulation is of the same kind as the relation between growth potentials and growth circumstantials explained above (p. 21 f.). Just as the growth potential defines a certain basic level within which growth rates can assume an infinite number of actual values depending on the environmental conditions, so differentiation defines a *specific level of reactivity* rather than a final reaction of the cell; a variety of manifest characters is then produced on the same level in response to the variety of prevailing conditions. This makes it imperative to think of differentiation in terms of potentialities rather than realities.

The principle of exclusivity of differentiations.

The principle of *discreteness* of differentiation, stating that differentiation is bound to proceed along definite and distinct lines, partially includes another principle which may be designated as the *principle of exclusivity.*

Although we have acknowledged the fact that a cell never produces anything half way between a nerve fiber and a muscle fiber, or between an intestinal digestive cell and a red blood cell, it is still conceivable that some intrinsic subdivision might occur within a cell allowing one part to differentiate in one and the other part in the other direction, producing not a hybrid but a chimera between cell types. In a sense this is the case in the higher forms of Protozoa in which specific mechanisms for contraction, conduction, digestion, excretion, etc., coexist within the same cell body. It can be easily recognized, however, that the higher an animal ranks in the evolutionary scale, the less of this inner versatility do its cells reveal, and in the higher Metazoa cells have evidently lost the ability of applying themselves to two specialized jobs at a time. There are only a few exceptions to this rule, and on the whole it can be maintained that *once one type of differentiation has taken possession of a cell, such cell can never become engaged concurrently in any other type of differentiation.* The cell in differentiation acts as an entity and as such concentrates on a single issue, unable to divide its faculties, energies and implements, to serve more than one performance. Consequently, differentiation initiated in one direction automatically precludes differentiation in any other direction. This is what we mean by the principle of *exclusivity.*

The principle of genetic limitation of differentiations.

Finally, a third principle can be established which in a sense is nothing but a more stringent formulation of the principle of discreteness and which may be called the principle of *genetic limitation.* Not only are the courses of differentiation which are open to a cell restricted to the definite number of established histological lines such as muscular, nervous, cartilaginous, etc., but a cell in effecting these differentiations is bound to follow strictly the peculiar ways and methods typical of its parent species. A muscle cell of a species *A* not only possesses the general characters common to all muscle (fibrils, excitability, contractility, etc.), but also bears certain marks which distinguish it as a member of species *A* from muscle cells of species *B*, *C*, etc. The species differences may consist of nothing more than different size of nucleus (p. 131), different amount and proportion of cytoplasm, different shape of the cell, different threshold of reactivity, different inclusions (granules, pigments), different behavior upon stimulation, etc., but they may occasionally go so far as to permit a cell of species *A* to form something which the homologous cell in *B* cannot form. Apparently if a cell sets out to form a muscle fiber or a nerve fiber or any other specialized structure, it can do so only in *the fixed and approved way generally practiced by that species.* In order to prevent misunderstandings, it must be mentioned, however, that this strict species-specific limitation applies only to the special manner in which a cell pursues a given course of differentiation, but not to the factors which make it enter one course rather than another. These latter, as will be shown later, transcend in their effects the limits of the species (p. 363).

Summarizing the principles of differentiation.

In conclusion, we obtain the following picture of differentiation. Cells emerge from the act of segmentation of the egg with morphological and functional characteristics which are by far more primitive and generalized than those found during later stages. The cells then continue to grow, to divide, and to differentiate with a pronounced, though not absolute, antagonism existing between mitotic activity and cellular differentiation. When a cell enters into one of the several discrete lines of differentiation available to the species, it does not pursue the whole course in one uninterrupted stride. It reaches several intermediate levels of differentiation where further progress can be interrupted for varying periods of time by renewed mitotic activity. Many cells remain permanently arrested at such lower levels, and only a certain proportion attains the highest possible degree of differentiation. Any level, including the highest, presents the cell in a condition of plasticity — "modulation" — the extent of which varies in different lines of differentiation. It permits the cell to react specifically to a variety of circumstances and to fluctuating conditions of the internal environment of the body (e.g., hormones). The manifest differentiations reached in the course of cellular development include changes of size, shape, motility, internal structure, production of specific substances, influences upon other cells and upon the surrounding matrix, and changes in general and specific reactivity. According as these characters change, the diversity of the parts of the body is progressively increased.

ORGANIZATION

The orderliness of development.

If the capacities for specific differentiation were distributed over the whole germ at random, the resulting structures and functions would be correspondingly haphazard. Simple growth increases the mass of the body, differential growth permits it to assume shape different from a sphere, and differentiation enables it to establish specific inhomogeneities on an increasing scale; but all these processes superimposed do not tell the full story of development. They explain that *some* shape and *some* internal differentiation is attained. However, the organism does not have merely *some* pattern; it has a *definite* and *typical* pattern. A pile of sand has pattern, too; its elements are different in size, shape, color, composition, and arrangement, constituting a variety which is no less complex than that of diverse parts within an organism. Yet, there is no regular grouping, no regular assortment of these characters, and no two piles show any resemblance in the distribution according to which the diverse elements are grouped. There are no inter-relationships among the elements other than those resulting from the fact that they are all subjected to the same laws of gravitation, friction, and mutual impenetrability. Now, an organism is not a pile. Its parts are not simply diverse in some way. What makes an organism an organism, is that the diverse portions are *definitely grouped and arranged,* maintain *specific mutual relationships,* and conform to a pattern which is *essentially identical* for all members of a species. In other words, a definite order prevails in development which prevents the complexity from becoming confusion, and the diversity from becoming chaos. This order according to which *every part is put into its proper place, and into specific relationships with other parts,* and according to which *the activities of every part are made to comply with the plan of the whole system* to which it belongs, is called *organization.*

In short, it amounts to the fact that the cat has a window in its pelt just where the eye looks out, and has the teeth in the place where the food is taken in. If we turn back to our list of differentiated characters observed in the organism, we note that not one of them could actually assume complete independence of the others without upsetting the harmony of the total pattern whose integrity is vital for the existence of the organism. Size, shape, arrangement, mutual connection, mutual effects and interactions, proportions, activities, etc., must interlock in a unique way from which there can be no major deviation if the developed organism is to survive. The developed organism, however, can be viewed as a cross section through a cable of developmental processes. An eye is nothing but the terminal stage of a set of eye-forming processes; a tooth merely a mark of the progress attained by a series of tooth-forming processes. In this light, the static organization of the formed body referred to on page 62, is simply the most recent stage of the dynamic organization which has governed its formation.

Degrees of organization.

Any stage of development discloses some degree of organization through which the relationships of the innumerable constituent processes of development are coördinated. To distinguish between different degrees of organization seems imperative for the following reasons. The organism contains organs arranged in a typical pattern. This expresses the organization of the *body*, in which the organs figure as units, irrespective of their intrinsic character and composition. The community of organs would have to be called organized even if each unit were in itself unorganized or homogeneous. As a matter of fact, however, an organ does possess an organization of its own; it can be recognized as a regular assemblage of units of a lower order — tissues — in typical arrangement and relationship. Again, as far as the organization of the individual organs is concerned, it makes no difference whether the constituent tissues are uniform and homogeneous or not. However, as we know, they, too, are organized; they consist of elements of the next lower order of magnitude — the cells — in definite arrangement and configuration. And, again, each cell is in itself an organized system composed of orderly interacting constitutents — nucleus, nucleolus, Golgi net, mitochondria, etc. In this view the body appears as a *complicated system of hierarchies of different orders of magnitude* each level of which has its own mode of organization.[1] Degree of organization, then, refers to the number of organized levels present in a system. In an early stage of development, for instance, there is organization at the higher level of the organism while there is no recognizable organization at the lower level of the organ.

When organization is conceived in the *dynamic* sense, as applying to *activities* rather than to *achievements*, then it becomes obvious that the orderliness of the *spatial* characters is only one side of the problem of organization. Activities are happenings in space *and* time. Therefore, if it is a question of definitely controlled activities, the control must extend as much to *temporal* as to *spatial* characters. In other words, organization means not only an orderly *spatial* pattern (size, localization, proportions, connections, etc.), but also definiteness of the *time* characters (sequence, phase, rate, duration, etc.) of development.

THE PROBLEMS OF DEVELOPMENT

The task of embryology.

The task of Science is to describe the phenomena of this world in the most truthful, complete, concise, consistent, and intelligible manner; or, in other words, to become acquainted with the world and to learn to understand it. The aim of embryological science is to perform this task for the phenomena of development. The phenomena of development have been outlined in the foregoing pages. There we find an utterly abbreviated story of development containing answers to the questions: *what* happens *where* and *when?* Thus we have become familiar with the object of our study and have discharged the first part of our task as scientists. The second and far more arduous part is still outstanding: we must reduce the story to its last elements and discover the reasons for its inner coherence; we must elucidate the basic order in the confusing fabric of events, and find out its underlying laws. Convinced of the fundamental unity of nature, we must, eventually, relate this order to that disclosed in other fields of Science. In short, we must find the answers to the questions: *how* and *why* do things happen as they do? We must *resolve the story of development into a minimum of simple and irreducible propositions* which will permit us, if not to predict in detail, at least to understand in principle, the infinite variety of specific happenings. To establish *general laws*, of which "individual cases" and "particular events" appear merely as casual expressions, thus becomes our prime concern. They constitute our *fundamental* problems. But approach to the fundamental problems is gained only through the solution of innumerable smaller problems that lie closer to the surface; although narrower in scope, they must be settled before we can penetrate to deeper and more ponderous issues.

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[1] *Heidenhain*, 1923.

Problems of development.

The problems of development present themselves automatically when we page through the list of developmental phenomena. Behind every phenomenon which arouses our curiosity but cannot be adequately explained in terms of familiar knowledge, there lies a problem. Any regularity, similarity, correspondence, or other connection among individual phenomena, as well as the absence of such relations in instances in which our logical train of thought would have expected them, hides a problem. For instance, cleavage is common to all developing eggs; so is differentiation. But are they interdependent? And if they are, which one controls the other? If they are not, what causes their concurrence? And is this concurrence, after all, essential for development? And, if so, for what parts of development? And, after having ascertained the answers to these questions, how generally do they hold? Can results obtained in one form be extended to other forms? If so, within what limits? If not, why not? And thus, one question brings forth another, *ad infinitum.* Some problems are entirely empirical; that is, in order to solve them, one has to go and try. Others can be solved by mental operations, by comparison and deductive thinking. Still others are sham problems formulated on mistaken premises and altogether incapable of solution. Many are solved only to give birth to a flood of new ones.

In this light, any attempt to list and classify problems of development in detail would seem to be of little sense and value. Nevertheless, a few general comments are in order.

Principles and mechanisms.

The problems of development are essentially of two kinds: those pertaining to *principles* and those pertaining to *mechanisms.* This distinction needs to be explained, and specific examples will probably serve this purpose best. One speaks, for instance, of the *principle* of "competition." One does not have to know the nature of the competitors to understand the rules of their conduct and the results of their actions. Competing for control in a common field, each expands as far as the others let him; they check one another according to their relative strengths. Strengthening one is tantamount to weakening the others. Strengthening or weakening all proportionately, leaves their balance unaltered. If their common domain is widened, they again apportion the new fields in proportion to their powers, and so forth. These phenomena are invariably the same whether the competitors are nations, tradesmen, hunting animals, growing plants, cells in a common nutrient, or whirls in a common pool. The *mechanisms* vary: nations wage wars, tradesmen use persuasion, animals deplete the stock of prey, plants exhaust the soil, cells withdraw nutrient from the medium, and one vortex diverts water from the next by centrifugal force. To know these *mechanisms* in no way adds to our insight into the operation of the *principle* of competition. For reasons of this kind it is necessary to draw a line between the search for principles and the exploration of mechanisms. Many events become fully intelligible only in terms of the principles which they obey, regardless of the mechanisms, often obscure, through which they are effected.

Rhythmical or wave actions are another case in point. Phenomena of resonance, interference, and diffraction can be predicted, controlled, and explained without giving a thought to whether one is dealing with the motion of water, sound, light, or electricity. Similarly, we speak of a general *principle* of "friction" which applies equally well to the braking mechanism of an automobile wheel, to the ease of flow of a liquid along the walls of a tube, and to the forces of economic and social maladjustment. We recognize principles of "conservation of energy," "minimum effort," "least resistance," "diminishing returns" in physics as well as in biology and economics, and everywhere they operate in precisely the same manner.

In this sense, we direct part of our endeavors to the study of the *principles* of development and the rules according to which these principles operate. Part Three will deal with these studies. We shall learn of a principle of "*emancipation*" or "*segregation*" and follow its effects, without having so much as a hint as to how it may be effected. We shall hear of a principle of "*induction*," as the unilateral determination of the fate of one embryonic part by another, but there will also be proof to show the great diversity of "inductive" mechanisms.

Such principles provide us with a few basic formulae and rules which describe and explain the conduct of a developing system in a coherent and consistent manner. They reduce known facts to a common denominator, permit correct predictions and preclude false presumptions. But in all this the existence of those principles is simply taken for granted without further analysis.

Analysis.

It is a further step to inquire into the *mechanisms* involved in a given phenomenon; to investigate the nature of the agents concerned, their mode of operation, and the causal links connecting the events. Using the example of the nervous system, Part Four will illustrate how problems of mechanisms can be tackled. The procedure is analysis by degrees. First, the complex phenomena are decomposed into simpler and elementary processes of biological order. If there were no other phenomena in this world but biological ones, the analysis would end here. As there is an inorganic world, the biologist must proceed further and elucidate the relation between biological and anorganic processes. He must attempt to trace the roots of biological processes into the known realm of physical and chemical phenomena. How successful he will be in this attempt, whether some day it will be possible to reduce *all* biological phenomena into such physical and chemical elements as have become known from the field of the nonliving, it would be idle to predict. While our advancement toward this goal perhaps seems fast to those who measure progress in terms of achievement, it must seem almost negligible to those others who view it in terms of the distance left to be gone. At present, the sensible attitude is neither overconfidence nor resignation but steadily continued work with an unbiased mind free from preconceptions.

Hierarchy of organizations.

One of the essential properties of an organism is that, complex as it is, it is, nevertheless, an *entity* with a considerable measure of *stability*, *durability*, and *organization*. Inasmuch as every animal organism is of macroscopic or microscopic, at any rate, supramolecular dimensions, the problems of this organization are not sheer problems of the constituent atoms and molecules taken as *individual* elements, but rather a matter of their *collective* behavior. Their specific interactions, groupings in the mass, mutual restrictions and activations, and so forth, lead to the emergence of new properties and rules which dominate the collective without being recognizable in its elements. Thus, the level of organization of an organism is infinitely higher than that of its component atoms.

But there are intervening steps over which this higher scale of organization is reached. The order of the organism is of the *hierarchical* type where units of any given level are subordinated to factors of the next higher level, are coördinated among one another, and are superior to the units of the next lower level. Thus, mounting from the atom which in itself is an organized compound of units of still lower order (electrons, etc.), we pass successively the levels of the molecule, the molecular aggregate, the micell (ultramicron), the cell, the tissue, the organ, the organism (see p. 103); and beyond lie the supraorganismal entities: colonies, states, and species. There is order at each level and there is order in the relations of the various levels; one might call the former "horizontal" and the latter "vertical" organization, to indicate their different character. Consequently, an entity of any level is composed of units of the next lower order of magnitude which are both coördinated and interrelated among one another and integrated into the whole which collectively they constitute.

This state of affairs calls our attention to the general problem of *the relation between the whole and its parts,* as one of the fundamental problems of the developing as well as the developed organism. Much of our further discussion will be devoted to this problem implicitly, and all theoretical concepts of Embryology have taken an explicit stand on it. In it lie the roots of the old controversy between the schools of *Preformation* and *Epigenesis*, most emphatically disagreeing on the relation of the parts to the whole in development.

Preformation and Epigenesis.

Preformationists maintain essentially that there is preformation of parts in the organism from its very first beginning; that these primordial parts are no less diverse for the fact that their differences are less ostensible during earlier stages of development; and that development consists fundamentally of nothing but a gradual conversion of latent into manifest differences without attending increase of complexity or emergence of new properties. According to this view, the organism as a whole appears as a product of *secondary integration* in which *primarily independent and individualized parts* join to compose a working unit of higher order.

Epigenesis, on the other hand, presumes that developing systems start from a rather primitive, homogeneous, chaotic or, at least, lowly organized condition into which increasing complication and real, not only apparent, diversification come in progressively as development proceeds; according to this view, the later parts of the organism are not as such pre-existing in the germ but are gradually established by a process of *individualization* within the *primarily integrated whole* of the forming organism.

As they are formulated here, the preformationist and the epigenetic interpretation of development appear as mutually exclusive. The attempts to decide the issue in favor of one or the other alternative have stimulated a great deal of valuable work, but no decision in favor of one view to the exclusion of the other has been obtained. As we now know, the reason for this failure has been that the problem was incorrectly stated. We know that "development" is not a single, uniform event; hence, there is no justification in our asking for a sweeping decision as to whether "development" proceeds according to the preformationist or the epigenetic formulae. As will be shown in Part Three, both viewpoints are correct and again incorrect, depending on the particular phase of "development" on which one chooses to focus.

Many problems of major and minor import have suffered a similar fate. They were stated in terms of clear cut alternatives, only to be disavowed by later experience which showed that where one had expected a decision of an "Either-Or," the actual answer was a "Neither-Nor." Taking a lesson from this historical fact, we have desisted for the purpose of this book from formulating the problems of devlopment in sharp and rigid questions to which equally well set answers should be expected, and have, instead, chosen to let the problems develop themselves, as we go along. After all, much of our scientific progress consists not so much of solving problems as of setting wrong problems right.

The organism as a system.

It will facilitate the understanding of the dynamics of development and prevent certain irrational problems from raising their heads, if one bears in mind one fundamental fact: that organisms are *systems* in the full meaning of the term. What is a *system?* Briefly, we can define it as a natural object that exists and preserves, or at least tends to preserve, its state and character by its own intrinsic forces. The converse of a system is an *aggregate* or a pile. Both consist of parts; but they differ with regard to the degree to which these parts are interdependent. While one can change or remove parts of an aggregate without altering the condition of the other parts perceptibly, the parts of a system are in such permanent interaction that whatever affects one part, involves all others, too. *None of its parts is independent, and the condition of each is determined by the character and the condition of the whole system.*

The *steady state* of a system is characterized by the *equilibrium of its intrinsic forces.* As soon as an external interference disturbs the existing balance, these forces react in the direction of restoring the original balance. Thus a system *responds to every change from the outside by a reaction of opposite sign from the inside.* The conservative forces of the system offset the external forces impinging upon it. In other words, the forces of a system act in such manner as to preserve the state of the *system as a whole* throughout the fluctuations to which its constituent parts are continually subjected. Where a mere aggregate would fall apart with no sign of inner coherence, a system obstinately tends to retain, and return to, its original configuration. Accordingly, the behavior of a part is not solely determined by its *own* properties but is controlled and directed by the *system as a whole.* This fact is a fundamental trait of physical nature, and the properties just outlined are as characteristic of anorganic as of organismic systems.

Anorganic systems.

As an example of a *mechanical system* we choose the *elastic body.* When its inner forces are in equilibrium with the external conditions, it possesses a definite shape. As soon as we change the external conditions by applying stretch, tensions appear in its interior which counteract the strain and, after the stretch has been released, bring the system back to its previous shape. Since the magnitude and orientation of the resulting stresses correspond to the intensity and direction of the strain, one can see that the systemic forces precisely match the foreign forces. Or take a *drop of mercury* on a plate. Its loaf-like shape is a compromise between its weight tending to flatten it and surface tension which tends to shape it into a sphere. Determined by the equilibrium between these forces, the shape of the drop remains constant. It restores itself after every deformation, and it remains unaltered when the drop is stirred. Bisecting the drop produces two drops of identical shape. Two drops fused form a single large drop. In none of these reactions can any individual particle of the system be singled out as responsible for the effect. We shall encounter many similar situations in the developing embryo.

Reversible bimolecular reactions may serve as an example of *chemical systems.* A reaction of the type $A + B \rightleftarrows AB$ reaches an equilibrium when all three substances are present in a definite ratio. Their relative concentration is maintained automatically by the properties of the system. If we increase the concentration on the left side of the equation, increased synthesis raises the concentration on the right side automatically; and if we add some of the reaction product there follows increased decomposition until the initial proportions are regained.

The cell theory.

These examples could be augmented indefinitely. They keep us from regarding the systematic behavior of organisms in vitalistic light. The point to be stressed, however, is that organisms do fall in the class of systems and, as such, naturally exhibit the general characters of system behavior. This realization also clarifies the role of the individual cell in development. The *cell theory* has emphasized the discreteness of cells, which often was taken to imply independence. Thus, the cells were compared to the bricks of a building, and development was considered on the same level as bricklaying. But as our knowledge grew, it became evident that neither the development nor the functions of the organism can be understood solely on the basis of *elementary* cell activities. More and more the particular mode of interaction and mutual control of the cellular elements commanded our attention. Gradually the emphasis placed on the individual cell as an isolated and autonomous element dwindled, and the conception of the organism as *a system in which the individual cells assume subordinated parts* gained ground. In Part Three it will be demonstrated to what extent this conception has been sustained by experimental evidence.

PART TWO

THE METHODS OF STUDYING DEVELOPMENT

HISTORY OF THE EMBRYOLOGICAL METHODS

The descriptive stage.

The array of methods made serviceable to the study of development embraces practically the whole range of biological methods in general, and like these had to pass through the three developmental stages typical of all biological history [1]: the descriptive, the comparative and the analytical stage. In the beginning there was pure *description;* data were collected and compiled in a drive to accumulate factual knowledge, partly in order to substantiate preconceived notions, partly simply to satisfy curiosity. Interpretations of the collected facts, if indeed attempted at all, were decisively influenced by tradition, prejudice, and the spirit and cultural philosophy of the age, and were remarkable for the prematurity of their generalizations.[2] Naïvely erected upon an altogether inadequate and too narrow foundation of facts, scientific theory at that stage resembles an inverted pyramid, narrow in its contact with the solid ground and expanding upward toward lofty speculation. Handicapped, retarded, and vitiated though the collection of pertinent data about development may have been by overgrowth of theory, nevertheless, knowledge of facts kept steadily growing, and soon the need for comprehensive classification and correlation of the many individual data made itself felt.

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[1] Lack of space precludes our presenting detailed data on the history of embryology. Instead, we bring the following terse synopsis. In reducing history to such a minimum of rudiments, one cannot avoid schematic oversimplification. It should be understood, therefore, that neither were the different phases in the evolution of our science so sharply delimited, nor were their motives ever as patent as it appears from this condensed outline. For a comprehensive discussion of the history of embryology, see *J. Needham*, 1934.

[2] Heated arguments, often more dialectic than scientific, mark this era. Antagonistic schools of thought were irreconcilably arrayed against each other: *Ovists* (asserting the primacy of the egg) vs. *Animalculists* (conceding monopolistic role in development to the spermatozoon); *Preformationists* vs. adherents of *Epigenesis;* believers in *Spontaneous Generation* vs. advocates of *Continuity of Life;* etc.

The comparative stage.

Thus our science entered into the second stage, that of *comparison.* Facts were aligned and classified according to similarity and dissimilarity, relationship or remoteness. Instead of continuing to build numerous hypotheses and generalizations upon but a few facts, one began to reduce the multitude of known facts to a few common principles as common denominators. The pyramid, with its factual foundation growing broader and steadier, began to right itself. When, in the earlier part of the nineteenth century, the comparative method penetrated into embryology, it soon bore rich fruits. It was this method that led *Karl Ernst von Baer* to establish some of the most fundamental principles of embryogenesis; for instance, the conception of *germ layers* as transitory units of embryonic organization, and the principle of *gradual increase in the complexity* of embryonic structures. The rules arrived at by comparative studies of development were, at first, not committed to any particular interpretation. They were merely formulae bringing some degree of order into what formerly had appeared to be a chaos of incoherent facts, while no reason was offered why there should be such an order.

This situation changed suddenly when, about the middle of the nineteenth century, the then new theory of *Evolution* injected sense into the hitherto purely formalistic statements of embryology. According to the evolutionary doctrine, in which the adult forms populating the earth were represented as terminal branches of a widely ramified tree of phylogenetic development, the embryonic development of each form — ontogeny — was considered to be a miniature reproduction of that portion of the phylogenetic tree lying between root and end branch. Whereas *von Baer* had merely recorded as a fact the close resemblance between the embryonic stages of different forms, *Haeckel* interpreted that resemblance on the basis of a common past, proclaiming ontogeny as a true recapitulation of phylogeny. Relationship henceforth was interpreted as kinship, and the succession of stages in embryogenesis was explained as the recurrent reproduction of ancestral forms. No longer was the definite order of events in the embryonic history of the individual taken for granted; the question was asked as to why the embryonic stages are linked in the particular sequence in which they are found to be seriated, and the answer was given in terms of the past history of the species. For instance, the blastula and gastrula stages recurring in the development of practically every higher form were supposed to be each a replica, in a sense, of an ancestral form, common to all Metazoa; it was contended that every germ, by going through the blastula stage, gained, as if by memory, the capacity of transforming into the gastrula, and from there into the next stage, and so forth, retracing the steps of the evolution of the species. Gill slits were thought to make a temporary appearance in mammalian development for the reason that they had been adult features in the fish-like ancestry. Thus, recapitulation of phylogenetic history was acknowledged as an explanatory principle, and methods current in the study of *history* were applied to the field of embryology. Inasmuch as, reciprocally, the embryonic history of the individual was searched for evidence to document the history of species, the study of embryology was greatly stimulated by the impulse received from the evolutionary doctrine which throughout the second half of the nineteenth century held the center of biological interest.

The analytical stage.

During the last decades of the century, however, while this development of affairs was still in progress, embryology rose to its latest stage, the *experimental* and *analytical.* Whereas the historical viewpoint was represented chiefly by naturalists — zoologists and botanists —, medical science, to further her own aims, had begun to approach development from an essentially different angle. Confronted with the task of repairing functions and structures that had gone wrong in the human body, medical men could derive no assistance from the finalistic historical viewpoint which explained everything present on the basis of a distant past. They could not step back into that past to remove some ancient conditions that had permitted a certain weakness or tendency to abnormality to develop in the body; bound to deal with the emergencies arising under their eyes in living individuals, they could hope for remedies only provided the immediate causes of the disturbances were known and accessible to direct observation and intervention. Thus, it was largely men with medical interests, pathologists and anatomists and histologists, who indulged with increasing emphasis in a study of the organism not as a product of the past but as a product of the present. They could not possibly acquiesce in accepting such statements as that of the ontogenetic recapitulation of phylogeny as final explanations of embryonic events; only knowledge of the *actual causes, factors,* and *mechanisms,* normal as well as aberrant, operative in transforming the individual from the egg to the adult form could satisfy their inquisitiveness and bring them nearer to their goal: to understand the reasons for deviations from the normal course. *Dynamic causation* rather than *historical seriation* became the thread along which the embryonic events were lined up and linked. Just as in the exact natural sciences, physics and chemistry, so in biology, too, the tendency grew to explain every change on the basis of the prevailing conditions under which it arises; the aim became to describe and understand every state of a living system as conditioned by the immediately preceding states. Embryology thus entered its most advanced phase, that of *analysis.*

The experimental method.

Along with the new ideology embryologists adopted the methods of the exact sciences, particularly the *experimental* method. As a concerted and organized scientific endeavor, analytical embryology did not make its appearance until during the last three decades of the nineteenth century. While many authors contributed to its foundation, *Roux* was the one to give it a name and a program. He called the new discipline "*developmental mechanics*" and defined its aim as the analysis of the causes, forces and factors operating in the formation of an organism.[1]

It is obvious that the analytical viewpoint was not intended to replace the historical viewpoint, nor could it arrogate such monopoly. Analytical and historical approach complement rather than mutually exclude each other. Analytical examination divulges why an egg must become an embryo, and an embryo an adult, and an adult a corpse; while historical examination reveals why nature has realized out of the infinite number of imaginable possibilities only the limited variety which we find in actual operation. Accordingly, the conspicuous shift from the historical to the analytical viewpoint which embryology has witnessed during the last fifty years has been a change of focus and predilection rather than one of the subject and its merits.

Undoubtedly the analytical method owes most of its preferred position to its major tool, the *experiment*, whose application has led to decisions and conclusions of great definiteness, clarity and infallibility.

Purpose of experimentation.

What is an *experiment?* Broadly defined, it consists of an alteration of the conditions of a state or process, followed by a study of its consequences. The alteration may be accidental, as it is in many experiments of nature, or it may be deliberate as in laboratory or field experimentation. Nature presents us with situations of such a bewildering complexity and intricacy that an attempt at grasping and understanding them by mere observation seems utterly futile. The analytical experiment has been devised as a means of breaking them down into simpler elements. We single out individual components, test their effects or ineffectiveness, establish their qualitative and quantitative influences on other components, and rate them according to their significance. Consequently, the acknowledged function of the analytical experiment is to serve as an instrument *to illuminate and extricate matters with which the study of nature has acquainted us,* and *to solve problems which critical observation of nature has raised in our minds.* By remaining aware of this function, one can escape the obvious danger of letting experimentation become an aim in itself. Experimentation for its own sake, instead of in the service of the solution of problems, may be as legitimate a means of deriving happiness from creative efforts as is any other playful exertion of the profuse talents of mankind, e.g., artistic production. Occasionally it may even lead, and has led on past occasions, to important discoveries. And yet, from the standpoint of scientific utility and efficiency and of methodically sound procedure, aimless and random experimentation seems utterly wasteful and contradictory to the very spirit of scientific research.

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[1] *W. Roux,* 1912. Although publications dealing with developmental mechanics are widely scattered, the bulk of pertinent literature is contained in two Journals: Wilhelm Roux' *Archiv für Entwicklungsmechanik der Organismen* (formerly: *Archiv für mikroskopische Anatomie und Entwicklungsmechanik*), founded 1895; and the *Journal of Experimental Zoölogy,* founded 1904.

It has often been asserted that science is not an expression of the lavishness but of the economic tendencies of the human mind. Science is said to be engaged in describing, analyzing and correlating the phenomena of nature in the *most comprehensive manner,* yet, at the same time, in terms and formulations of *the greatest possible simplicity.* Our aim is to make the unknown intelligible in terms of the better known, and to establish order in the perplexing multitude of facts. Hence, the unceasing search for generalizations and comprehensive formulae to serve as time- and labor-saving devices — saving time and labor because they embrace in a single rule or law innumerable individual cases which otherwise would have to be studied individually. In his attempt to render more complete and intelligible the knowledge of the phenomena of nature, the scientist is faced with an alternative. He may patiently collect and examine individual facts, one by one, hoping that eventually he will be able to piece them together to form a coherent picture; or he may strive for a key position from which all those individual facts would be visible at one glance, so that he may be spared the necessity of groping for each one separately. It is obvious that the second procedure is by far more economic and advantageous, for the same reason that it is more efficient to blast a rock than to hammer it away in bits. From this angle it would appear, then, that the mere fact that an experiment has not been done before does not yet justify our doing it, unless there is a reasonable expectation that it will help to elucidate or simplify or correct our notions of natural things and processes.

The role of experiments in embryology.

In the study of development, the analytical experiment appears in two forms. One is the direct application of physico-chemical methods to the living organism; the other consists of altering the arrangement and the relationships of parts within the organism by surgical operations and similar procedures. In surveying some of these methods in the following pages, we do not aim at completeness; we merely intend to sample the principal procedures.

Remembering our postulate, that analytical experimentation should be planned *for the solution of problems,* and that it should turn to the *observation of nature* for its problems, we realize that *descriptive* embryology, histology, and pathology are the chief sources from which *experimental* embryology, histology, and pathology draw their problems. The experiment is useful even in purely descriptive research. Any interference, from the breaking open of the shell of a hen's egg in order to expose the embryo for prolonged observation, to the fixing and sectioning of tissues for microscopical preparation, constitutes an experiment, inasmuch as it changes the natural conditions. The ordinary determination of the physico-chemical state or of the chemical composition of an organism is likewise an experimental procedure, inasmuch as the methods of testing necessitate alterations, if not the destruction, of the living objects. Only by making suitable allowances for these artificial alterations are we able to reconstruct and describe to a fair degree of approximation the normal morphological, physical, and chemical stage of the object under investigation.

DESCRIPTIVE METHODS

Morphological methods.

The common anatomical and histological techniques have been appropriated to the study of the *morphological* details of embryonic organization. Many eggs are so highly transparent that their development can be followed directly under the microscope. Others require special treatment. Fixed embryos can be made transparent by imbibition with oil of a certain refractive index, much as a piece of paper becomes translucent in grease. The usual way of studying the interior of the embryo is, however, to section it into thin slices which are then mounted on glass slides. Treatment by this method, involving fixation, dehydration, staining, etc., entails more or less serious shrinkage, distortion, and modifications in the tissues, and the degree of such vitiation depends upon the agents used and the rate and care with which they are applied. In no case does the final preparation give an absolutely faithful picture of the living condition; cellular size, attachment, arrangement, inner structure, form — all these may have been affected. The fixed preparation on the slide can be as misleading in presenting aspects to which there is no counterpart in the living state, as in failing to reveal structures whose reality is well known from other evidence. Many controversies have arisen from these uncertainties of the method. Some authors were only too ready to accept the fixed histological picture in every detail as a true image of the living tissue, while others, going to the opposite extreme, distrusted it so thoroughly that they would not admit the reality of structures unless these could be demonstrated in the living organism.

To be sure, laborious and extensive studies comparing the effects of various fixing agents have tended to reduce the danger of deception to a minimum. That is, we have learned to recognize artifacts and to make proper allowances for the defects of the treatment. At the same time, however, the limitations of the method have not completely disappeared. An example of interest to the embryologist will make this clear. It concerns the problem as to whether the daughter cells emerging from the division of a mother cell become completely separated from each other or remain connected by means of fine protoplasmic threads, so called *plasmodesms.* Many a biologist was inclined to postulate plasmatic continuity among cells on the grounds that such a mechanism could account for the coöperation and coördination of the activities of cells during development much more readily than would mere contiguity. Protoplasmic bridges could conveniently serve as a means of transmission of messages or substances from cell to cell. Yet this is a question of facts, not of postulates, and histological studies were expected to yield a decisive answer. In this, however, they failed. There were some pictures that showed residual connections between the daughter cells; others were equally convincing in the opposite sense. In the former case, the presence of cellular connections might easily have been simulated by artifacts resulting from the histological procedure. In the latter case, delicate connections, even though present in the living cells, might have been broken by the sudden contraction of the cells during fixation. We know that many types of cells, e.g., blood cells or germ cells, are discrete bodies, moving freely and independently of other cells, but with regard to other tissues the question of protoplasmic connections between the individual cells is still a matter of dispute upon which even continued histological studies do not promise to shed much light. Its obvious limitations notwithstanding, the histological method represents still the main source of our information about the changes taking place during development in size, shape, arrangement, and constitution of the cells.

While formerly the various stains with which histological sections were treated served merely to make visible structures that were otherwise indiscernible, they have more recently also been used as *indicators* of chemical and physico-chemical properties of the treated cells — as microchemical test reagents.[1]

Chemical and physical exploration.

The *chemical* description of developmental stages is subject to limitations similar to those encountered by the morphological description. Chemical analysis of living systems is greatly handicapped by the fact that many chemical constituents of the organism change significantly in character when extracted from the living system. Despite many obstacles, however, conspicuous progress toward listing the chemical constituents of living developing systems has been made.

Determination of the *physical* conditions during development is again no simple task, for the very method of testing such characters as elasticity, mechanical consistency, electrical charge, compressibility, etc., frequently requires procedures that upset the finely balanced living state, thereby yielding information about artificial rather than natural conditions. For example, the *viscosity* of pulp can be estimated by determining the resistance encountered in stirring it up. In an attempt to apply this method to the determination of the viscosity of protoplasm, fine glass needles with

[1] See *Cowdry*, 1924.

tips measuring not more than a few thousandths of a millimeter were introduced into cells and moved therein so that the degree of their bending might give an idea of the degree of solidity of the content of the cell.[1] Soon, however, it was discovered that the very process of stirring changed the consistency of the protoplasm in the direction of greater fluidity. Hence, all that was gained was some knowledge concerning an experimentally altered state of the protoplasm instead of the normal one.[2]

None of the mentioned shortcomings render a method unfit for the study of living systems, and actually most of what we have learned about the morphology, physico-chemistry and chemistry of development has been secured in this fashion. But one should not lose sight of the fact that a method which, while aiming at exploring the living state, necessarily destroys it, can hardly be more than approximative. Preference must be given to methods permitting the examination of the living state with the least possible interference.

The study of cells in the living.

The study of living cells in *ultraviolet light* and in the *dark field* (see example 13) has revealed structures that would not have been visible in the living under the ordinary microscope. The use of *polarized light*[3] made it possible to study the oriented arrangements of elements of submicroscopic dimensions. The application of *X-rays* has enabled us to carry these studies into the range of molecular dimensions,[4] inasmuch as the analysis of the diffraction patterns obtained from tendons, nerves, muscles, exposed to X-rays, revealed crystalline regularity in the grouping of the constituent atoms. Since the specific arrangement of molecules and molecular groups appears to be of fundamental significance in development, the prospective value of these optical techniques can hardly be overrated.

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[1] *Chambers*, 1924.

[2] The subject has been reviewed by *Heilbrunn*, 1927.

[3] *W. J. Schmidt*, 1924, 1938.

[4] *Astbury*, 1933; brief review: *Sponsler*, 1933.

The study of developmental kinetics.

All the methods touched upon in the foregoing pages have in common a static aim: the exploration and description of an egg or a cell or a tissue or the whole embryo at a given stage. Development, however, signifies transition from stage to stage; thus it is the changes in which embryology is primarily interested, and methods had to be invented to follow and study them. The most common but also the most indirect method is that of reconstructing the whole line of development by comparing a number of stages selected at suitable intervals. The process is one of interpolation and resembles that of drawing a curve of which only certain points are known. The more points there are plotted, the less arbitrary the course of the curve becomes, but there is always some subjectivity left in it. The same holds true for the attempt to trace embryonic development by studying a number of stations through which it passes. Unless the intervals between the stations are practically infinitesimal, imagination has to fill in where actual observation is wanting.

Cinematography.

Considerable progress has been made in recent years in the devising of methods for *direct* observation of the course of development. *Time-lapse cinematography* brings into the range of our immediate perception phenomena that otherwise would not be perceptible because of their extreme slowness. By taking individual exposures of the developing object at regular intervals (several seconds to minutes) and later projecting the film at normal speed, i.e., sixteen frames per second, naturally slow processes can be speeded up on the screen to any desired rate. Most instructive pictures have thus been obtained of the development of chicks, fishes, amphibians, and others; furthermore, records of cells in mitosis, in phagocytosis, in tissue formation and disintegration, of the development and regeneration of nerve fibers, of the repair of injured muscles, and so forth. Many interesting facts that had formerly been overlooked were brought out by this method, as, for instance, slow periodical contractions and waves agitating the surface of developing eggs.

Methods of marking.

The great resemblance among different embryonic cells aggravates the task of tracking local groups through their developmental career. Considerable and significant shifts of materials relative to each other take place during earlier embryonic stages without our being able to discern them, because in these stages all parts look so very much alike that none of them can serve as landmarks by which to judge the shifts. Some eggs possess natural marks, for instance, pigmented spots, but most of these disappear too soon to be of great assistance. One has, therefore, taken recourse to the application of artificial landmarks: one tried to tag cell groups by marking them with some durable stain.

Colored marks.

The process is called *vital staining.*[1] Its success hinges on several conditions. First, the stain applied to a localized region of the germ must stay localized without diffusing into the vicinity. Second, the stain must remain visible for the desired length of time without fading. Third, the stain must be nontoxic and harmless so as not to destroy or even impair the vital activities and embryonic performances of its carrier. Several stains were found to satisfy these conditions: for instance, *nile blue sulphate, neutral red,* and *Bismarck brown.* Small pieces of agar are soaked in a solution of the reagent and then are applied to the surface of the cells to which they transmit the stain. The marks are conspicuous enough to allow cells thus distinguished to be traced during subsequent migrations even into the interior of the germ. The method has been successfully applied to echinoderms, ascidians, fishes, amphibians, and birds.

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[1] This method has been elaborated for amphibians by *Goodale,* 1911, *Smith,* 1922, and *Vogt,* 1925; for birds by *Wetzel,* 1925; for echinoderms by *v. Ubisch,* 1925, and *Hörstadius,* 1928. It has been widely used ever since.

Other marking methods.

Another method to make local cell groups stand out from their neighbors is to replace them by cells of *different character*. Frequently germs of closely related races or species differ in the amount of *pigmentation*. Instead of staining unstained cells with a vital stain, one can simply substitute for them a corresponding group from a naturally pigmented race (Fig. 16).[1] *Size of cell nuclei* is another tag useful in

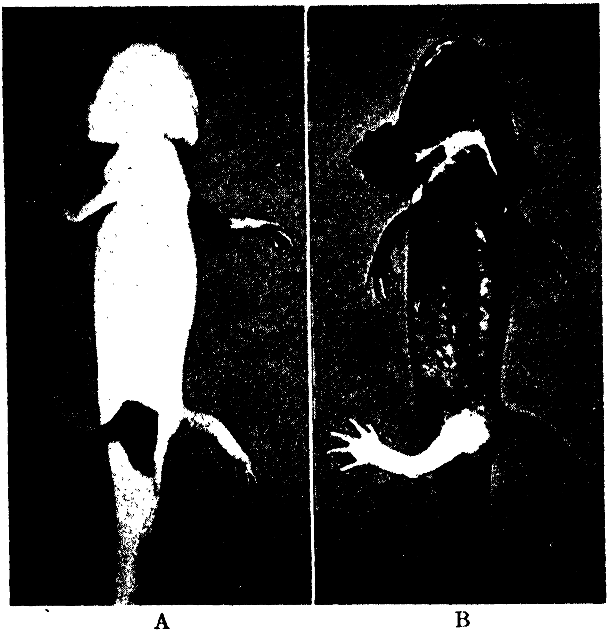

FIG. 16. Retention of coloration by transplanted tissue. (After *Ruud*, 1929) Cross transplantation of embryonic limb buds between specimens of the white and the dark-colored race of the Mexican Axolotl. The transplanted buds have differentiated into limbs (T) of the coloration of the donor. ×7/10.

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[1] Originally applied to mark the outgrowth of cells from one partner into the other in a graft combination of amphibian larvae (*Harrison*, 1903, see Fig. 28), this method has been widely used, particularly by *Spemann* and his school (see *Spemann*, 1938). Cf. Fig. 61.

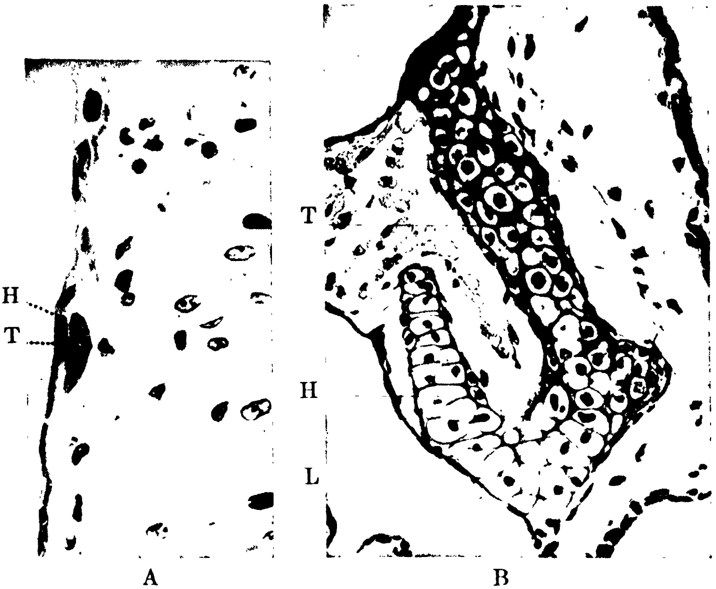

FIG. 17. Identification of the origin of grafted cells by the specific size of their nuclei. Transplantation of neural crest (see p. 510) of an *Axolotl* embryo into a *newt* germ (*Triton taeniatus*, neurula stage). (From *Raven.*) Axolotl nuclei are distinctly larger than newt nuclei.

A, a cell derived from the graft with large nucleus (T) lies in the cutis next to an epidermis cell of the host (H). × 420.

B, Jointly with host material (H), grafted material (T) has produced head cartilage. Note the sharp boundary (at L) between graft and host cells indicated by the sudden change of nuclear size. × 165.

the trailing of cell groups; if two species differ consistently in nuclear size, as they often do, cells transplanted from one into the other remain permanently distinguishable by this character (Fig. 17).[1] The methods allowing such an exchange of materials by transplantation will be discussed below. It is, of course, prerequisite for the success of either of these methods that the critical characters of the transplanted cells (pigmentation, nuclear size) be ineffaceable and unmodifiable. Natural color marks and nuclear characteristics have been demonstrated to answer this demand to all practical purposes (cf. p. 310).

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[1] *G. Hertwig* (1925) exchanged material between diploid and haploid individuals in amphibians. The haploid individuals were produced by inseminating normal eggs with radium-treated sperm; as a paralyzed sperm nucleus does not take part in development (see p. 158), all cell nuclei remained haploid and of half normal size. *Raven* (1936) exchanged material between species of different nuclear size.

Growth records.

Moving pictures and vital stains procure *continuous* records of embryonic events. For most purposes of embryology, however, *discontinuous* samples are sufficiently representative of the whole course. Thus, studies of growth are based on sampling. By plotting the size or weight of an organ or of an organism against time, one obtains *growth curves.* Such curves not only enable us to extrapolate size or weight at any given moment during the intervals separating the actual measurements, but they also make it easy to grasp at one glance the character of the whole growth process. All our information about growth, as summarized in Part One, has come from "descriptive" studies of this sort. Although growth curves are primarily convenient means of compiling empirical data, more ambitious use can be made of them, when one regards them as expressions of underlying general laws of growth, much as one regards the parabolic curve of a tossed stone as an expression of the fundamental laws of motion. The work on heterogonic growth reported above (p. 38) illustrates the fruitfulness of this approach. The gain from such studies is more than mere factual data; valuable insight into the very processes of growth and even some clues concerning the driving forces can be the rewards of a critical evaluation. As an example of the revealing character of growth curves we choose the embryonic growth curve of the chick.

Example 1.[1]

The embryonic growth curve of the chick.

The different organs of the chick embryo were carefully weighed on successive days of development, and the data so

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[1] *Schmalhausen,* 1926.

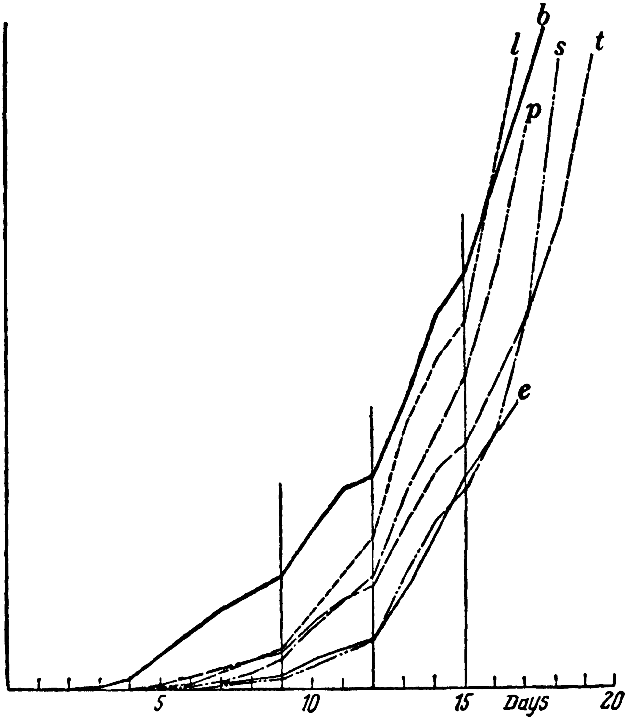

FIG. 18. Growth curves of organs of the chick embryo. (Constructed after *Schmalhausen*, 1926) Abscissa: Age of incubation. Ordinate: Weight of organ. For better comparison different scales were chosen for the ordinates of different organs so that the growth of small and large organs could be immediately compared.

l, Liver (1 cm. = 30 mg.)
b, Brain (1 cm. = 45 mg.)
p, Lung (1 cm. = 7.5 mg.)
s, Stomach (1 cm. = 135 mg.)
e, Limb (1 cm. = 200 mg.)
t, Total weight (1 cm. = 1.9 g.)

obtained were then plotted against time. A glance at the resulting growth curves (Fig. 18) reveals that growth does not proceed evenly throughout the embryonic period; there are characteristic inflections in the growth curve which occur too regularly to be ascribed to accidental fluctuations. One notes depressions of growth around the 4th, 9th, 12th, and 15th days.

At first sight the disclosure of these periodical retardations

> seems to contain nothing more than a statement of some interesting detail concerning the growth of the chick embryo. But if one tries to correlate this fact with other embryological data, it at once assumes more general significance. A survey of the progress of *differentiation* and *morphogenesis* in the chick embryo shows that these processes also advance unevenly,[1] and closer inspection has fixed the peaks of their activity about the 4th, 9th, 12th, and 15th days. Thus, maxima of differentiation coincide with minima of growth. From this, the conclusion has been reached that *acceleration of differentiating activity is attended by retardation of growth activity*, or, in other words, that there is some antagonism between differentiation and growth. For a further discussion of this point see page 85.

It will be realized from this example that occasionally real insight into principles of development can be obtained simply by thoughtful and intelligent evaluation of data obtained by purely "descriptive," i.e., observational, methods. Without being strictly "analytical" — because they can only suggest but neither test nor prove — these methods must be credited with important discoveries in embryology.[2] Nevertheless, for final and decisive checks, one must resort to the experimental methods proper.

EXPERIMENTAL ANALYSIS

General Remarks

The planning of experiments.

Every experiment comes down, in the last analysis, to a simple proposition: there is always a central object in which we are interested, and there is a number of conditions which we know or suspect to have a bearing on it; so we set out to study what bearing. The object on which we focus may be the whole organism, as opposed to its environment; or it may be any part of the organism as opposed to the other parts. Generally speaking, we study the relation of two conjugated variables, y and x, of a mathematical function, the dependent variable, y, representing our central object of interest, while x represents the variable factors on which y depends. We vary x arbitrarily to $x_1, x_2, x_3 \ldots$ and tend to establish the corresponding changes of y to $y_1, y_2, y_3, \ldots$ For instance, by measuring growth rates in various temperatures, one gathers information concerning the dependence of growth, y, on temperature, x; or by exposing an egg to various concentrations of a drug, x, one determines the tolerance, y.

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[1] *Schmalhausen* and *Stepanowa*, 1926.

[2] Measurements of the length of insect larvae at successive instars have, for instance, suggested that every cell divides just once during the interval. For, the quotient of longitudinal increase from one moulting to the next was found to be cca.1.26. Since this value equals $\sqrt[3]{2}$, one has concluded that the larvae grow proportionately in all dimensions, exactly doubling their volume during each instar (*Przibram*, 1922).

However, it would not have occurred to us to study *quantitatively* the effects of increased temperature on growth, or the varying severity of poisoning by a given drug, unless we had been aware in the first place that growth depends on temperature at all, and that the drug is poisonous. That is, a *qualitative* demonstration of the existence of a relation comes before detailed quantitative exploration. In the field of developmental analysis we are unfortunately still in the stage where most of our experiments must be devoted to the former, qualitative, task. We are still too busy with ascertaining what depends on what, to be able to accord to each established relationship a comprehensive quantitative treatment. Often when a certain phenomenon attracts our attention, we are at first at a complete loss to tell with what other factors it may be related. Patience, ingenuity, and, above all, *systematic procedure* are, then, needed to track down the pertinent factors among the bewildering number and variety of possible choices.

Never should the approach be purely hit-or-miss, but presumptions and expectations, born from careful observations and logical inferences, should give it its direction. Experiments are designed to test and decide issues; therefore, there ought to be an issue before one ever starts to experiment. Exploration should be guided by definite anticipations; no matter whether eventually they will turn out to have been right or wrong, they will have served us well as guides into the wilderness of the unknown.

Hypothesis as guides in experimentation.

Presumptions of this kind, built on fragmentary, but suggestive, data are called *hypotheses.* No experimental scientist deserving that name can do without them. Always one jump ahead of the facts, a hypothesis points to the next step to be taken in producing more pertinent facts. It constitutes a sort of mental scaffolding to be used so long as the facts are scarce, and to be abandoned as soon as actual facts have become available which either substantiate or contradict the original suppositions. In either case, the hypothesis has served as a stepping stone in our quest for knowledge. This *pragmatic* role of hypotheses and theories has long been recognized and appreciated; to quote an author of the year 1772 (*Priestley*): "Very lame and imperfect theories are sufficient to suggest useful experiments which serve to correct those theories, and give birth to others more perfect. These then occasion farther experiments, which bring us still nearer to the truth, and in this method of approximation, we must be content to proceed, and we ought to think ourselves happy, if, in this slow method, we make any real progress."

Hypotheses must be tested.

Hypotheses are of immense value, provided one does not confound assumptions with realities. Sometimes a hypothesis seems so self-evident that one feels inclined to accept it as correct even without experimental verification. This is an outright fallacy. For, our judgment is in no way immune to deception, and no assumption can be plausible enough to be exempted from experimental scrutiny. If a theory aspires to becoming incorporated into the foundations of our knowledge, it must be subject to tests as rigorous as those applied to the foundation of a building. Experience has taught us to take almost nothing for granted before having applied a critical test. Not a small amount of biological work has led to erroneous notions simply because it had relied on premises that appeared so logical and obvious that no further test was deemed necessary. Indeed, the field of biological phenomena contains a richer source of deceptions than perhaps any other field of science. The following cases may exemplify this.

Example 2.[1]

Unsuspected failures of plausible assumptions.

In the tadpole of the frog the fore limbs develop inside the gill cavities hidden beneath a large covering fold of skin. There they complete their development and become mobile, without being visible from the surface. Then, at the time of metamorphosis, they break through the covering lid of skin, emerging through a hole that has formed near where the elbow had pressed against the surface. In view of the extensive motility exhibited by these limbs before their perforation, it would seem quite natural to assume that the pointed elbows gradually wear a hole through the overlying skin and thus enable the delivery of the limbs. It required an experimental test to show that this almost obvious assumption is erroneous. If development of the limbs is suppressed by extirpating the limb buds in an early stage, the *skin over the limbless cavity ruptures* just the same, about the time when normally the limbs would have perforated. In other words, the limb has nothing to do with the formation of the hole. As a matter of fact, it appears now that the hole is caused by the resorption of the gills which takes place at metamorphosis, or by autonomous disintegration of covering skin.[2]

Example 3.

The secondary sex characters of the chick have been found to depend on specific substances, *sex hormones*, discharged by the gonads. The most spectacular effect is that on plumage. Not only does the plumage lose many of its specific sex characters in animals from which the sex glands have been removed, but such animals can even be made to assume some features of the opposite sex when they are treated with an adequate dose of the opposite sex hormone.[1] In view of the fact that other birds (ducks, pheasants) reacted similarly, one might have felt justified in accepting the gonad-plumage relationship as a principle of general validity. It came as rather a surprise, when the repetition of the experiments on still another group of birds (some finches) suddenly brought to light a form, the sexual dimorphism of whose plumage proved entirely *independent of the gonad*, and in which the plumage failed to change after the removal of the gonads.[2] Therefore, there is no uniformity in the plumage controlling mechanisms among birds.

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[1] *Braus*, 1906a.

[2] *Helff*, 1926, 1939; *Blacher*, *Liosner* and *Woronzowa*, 1934.

These two examples sufficiently support our point, that in biology even the most plausible assumptions need verification, and that generalizations are bound to remain tentative and hypothetical until their range of validity has been clearly delineated by a reasonable amount of experimental tests.

Need of objective standards.

If alert reasoning and mental audacity are valuable assets in entering an experimental venture, restraint and critical acumen must prevail in the evaluation of its outcome. Unconscious selection and biased rating of the results favoring those best in line with the experimenter's anticipations and preconceptions must be excluded at all cost. Deception by chance results can be avoided by conducting experiments in sufficiently great numbers under identical conditions. The significance of quantitative effects can be checked by the methods of statistical mathematics.[3]

Experiments performed by nature.

Large scale experiments on development are perpetually performed by *nature*. A host of instructive experiments,

[1] For a survey of the whole subject, see *Domm*, *Juhn* and *Gustavson*, 1932.

[2] *Witschi*, 1936.

[3] *Davenport* and *Ekas*, 1936.

pertaining both to the relation between the developing organism and its environment and to the mutual relations of the parts within the organism, is going on right under our eyes without any artificial intervention, if only we learn to recognize them. The whole field of *ecology*, for instance, is full of pertinent examples.[1] There we find recorded how different natural environments affect the development and the reactions of animals. So, for instance, the fact that animals develop faster in higher temperatures, has been known long before systematic temperature experiments were undertaken. It is obvious that the more complete such ecological records are and the wider range they cover, the more they approach the standards of real experiments, with nature providing us with the gradation of test conditions which otherwise we must produce deliberately in laboratory experiments.

Example 4.[2]

Effect of salinity on development.

Comparative studies of specimens of the small crustacean, *Artemia,* collected in waters of different brackishness revealed that *form and proportions of the body varied distinctly according to the grade of salinity* (Fig. 19). Thus, a correlation of the recorded transformations with the degree of salinity has furnished information concerning the effects of salinity on development. The information obtained from these observations was later confirmed and amplified by controlled laboratory experiments.

Similarly, differences in the appearance and characters of members of the same species, when reared in cold and warm climates, in light or dark surroundings, in more acid or more alkaline media, under oxygen-rich or oxygen-poor conditions, can actually replace experiments on the influence of temperature, light, acidity, and oxygenation on development.

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[1] *Hesse,* 1937.

[2] *Schmankewitsch,* 1875, and others; Figure 19 taken from *Abonyi,* 1915.

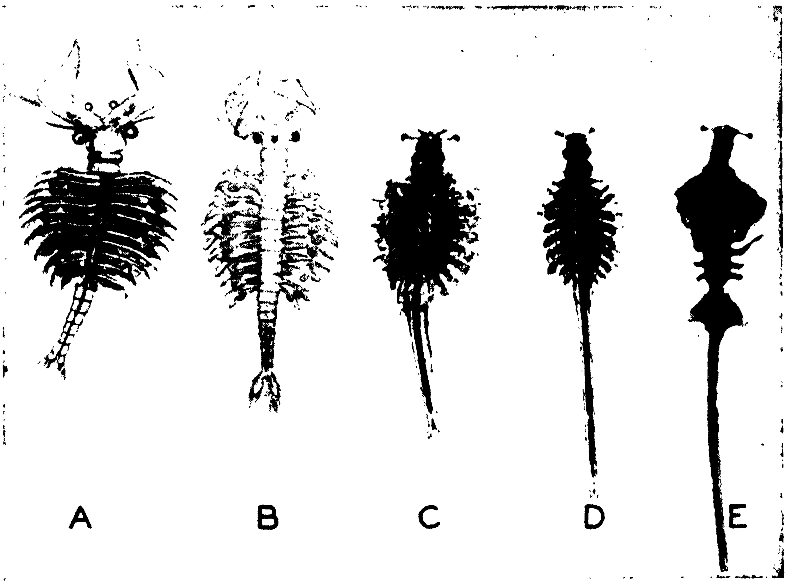

FIG. 19. Effect of salinity of medium on morphological characters of the crustacean, *Artemia*. (From *Abonyi*, 1915) A to E represent progressive modifications as the salinity of the medium increases. × 5.2.

A, sample from fresh water.
B, from very dilute salt water.
C, from salt water of specific gravity 1012.
D, from salt water of specific gravity 1090.
E, from salt water of specific gravity 1142.

Malformations as experiments.

Moreover, *abnormalities* and *malformations* arising under natural conditions may carry valuable evidence concerning the mechanics of development provided the conditions under which they have arisen are known.

Example 5.

Ctenophore development in troubled seas.

In collecting ctenophores, which normally possess eight rows of swimming plates, a zoologist[1] noticed that at certain times twin larvae would occur bearing only four rows; it occurred to him that this always happened after heavy storms when the sea had been stirred up, and, correlating the two facts in his mind, he ventured the assumption that the *deficient individuals had developed from eggs which had been broken in two* as they were tossed against rocks. This would presuppose, of course, that the capacity of the ctenophore eggs to produce rows of plates is strictly limited to the normal number of eight, so that half eggs would form only half the regular number. It was this observation which suggested the ctenophore egg as a presumably favorable object on which to test one of the fundamental concepts of embryology, that of preformation. As a matter of fact, a later experimental investigation in the laboratory has fully corroborated this experiment originally carried out by nature (see p. 236 and Fig. 41).

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[1] *Chun*, 1880.

Example 6.

Congenital defects in man.

Some human individuals are born with bodily defects, which are either inherited abnormalities or due to acute accidents during the developmental process. While these defects may often seem to be circumscribed, a thorough investigation will generally reveal that other structures are also affected, although possibly less conspicuously. Thus it has been observed that the absence of an arm or a leg at birth is accompanied by subnormal development of the corresponding nerve centers (Fig. 20).[1] This experiment of nature deserves credit for the

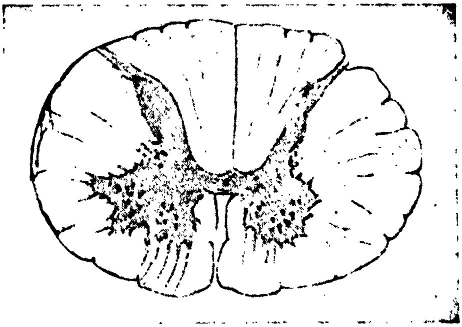

FIG. 20. Unilateral defect in the brachial region of the spinal cord of a human individual born with one fore-arm lacking. (From *Edinger*, 1921) The gray matter on the armless side (right half of the picture) is definitely undersized as compared with the opposite normal side.

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[1] Cf. *Edinger*, 1921.

discovery of a *developmental correlation between an organ and its nerve supply* — a problem to which a considerable amount of laboratory research has since been devoted (see p. 549 f.).

Example 7.

Abnormal growth and glandular function.

Some human individuals develop a remarkable abnormality characterized by excessive growth of jaws, hands, and feet, concomitant with other irregularities of the growth pattern. This is known as *acromegaly*.[1] By a careful check of other bodily symptoms attending this condition, a clue was found as to the cause of the disturbance; for the phenomenon proved to be associated with abnormalities of the *pituitary gland.* Thus, the discovery of the *growth-controlling effect of the hypophysis* is, in the main, the result of an experiment of nature.

External and internal factors.

On the other hand, just as it is exceptional for a physicist to wait until nature provides him with a crucial experiment, for instance, a solar eclipse, so the biologist does not depend on natural experiments to any great extent. His major source of information is experiments deliberately conducted by himself under conditions over which he has control. In our field of interest these experiments fall into two main groups, one devoted to the study of the *dependence of the developing organism as a whole upon its environment,* and the other aiming at the determination of *mutual interactions and interrelationships of parts within the developing system.* It is customary to call the factors involved in the former group *external* factors, and those in the latter group *internal* factors. For convenience, we may follow this convention although in practical application the terms often lose their distinct meaning. Many external factors must first become internal factors before they can act. We may simply point to nutrition as an illustration: food must first be digested, resorbed and transformed before it can contribute to cell growth.

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[1] Cf. *Van Dyke*, 1936.

The Study of External Factors

The study of the environment.

The part taken in development by the external environment is very variable. On the whole, one notes that the dependence is more marked in lower than in higher organisms; the maximum independence from environmental fluctuations is reached in the viviparous animals which develop inside the maternal organism. The effects of environmental variations are, therefore, best studied in forms most directly exposed to them, that is, those developing in an extraneous, inorganic medium. *Milieu* is a collective term for the entity of physico-chemical and biological factors surrounding a living system. There are manifold influences of the milieu on development, and some external factors assume greater prominence than others. In order to study their respective weight and effectiveness, we must try to dissociate them and test them individually.

The main factors calling for consideration are:

1. *Mechanical* factors (e.g., gravitation, pressure of the medium, tensions, currents, etc).
2. *Radiations* (e.g., visible light, infra-red, ultra-violet, X-rays, heat).
3. *Electrical* factors (e.g., electrostatic fields, currents).
4. *Chemical* factors (composition of the medium).
5. *Physico-chemical* factors (e.g., osmotic pressure, hydrogen ion concentration, viscosity).

We cannot strictly isolate these various agents because most of them are ubiquitous, but we can separate them in a relative sense by subjecting them, one at a time, to arbitrary changes, while keeping the others constant. Intensity, duration, and mode of application of the factor under scrutiny are varied, and the ensuing effects upon the organism are recorded. The methods of controlled dosing and timing are a matter of physical and chemical technique and fall outside the scope of this discussion. Only a few examples of specific applications to developmental problems may here be mentioned.

Mechanical factors.

The influence of *gravity* on the development of eggs can be studied by inverting the eggs, in which case their position in the gravitational field has been altered (see p. 347),[1] or by keeping them agitated during the period of development so that the establishment of any definite orientation is prevented.[2] These comparatively ancient embryological methods have now, however, been completely replaced by the use of the *centrifuge* (see p. 190). Centrifugal force is not only of immensely greater intensity than gravity but can also be conveniently graded. A modern *ultracentrifuge* allows eggs to be subjected to a centrifugal force several hundred thousand times gravity,[3] and clever optical devices make it possible to observe the eggs while under treatment (Fig. 32).[4] Special apparatus have been constructed to study the effects of a general increase in the *pressure* of the medium on developing eggs.[5] Localized pressure is applied by pressing eggs between plates or sucking them into tubes of smaller diameter (see p. 200).[6]

Temperature.

Raising or lowering the *temperature* level above or below normal not only permits to establish the range of temperature extremes (see p. 24) tolerable for development but has a more fundamental significance in revealing the *temperature coefficients* of developmental processes. It is known that the rate of ordinary chemical reactions in a normal temperature range is roughly doubled by raising the temperature level by 10° C (Q_{10}). If, then, a biological reaction —

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[1] *O. Schultze,* 1894; *Penners* and *Schleip,* 1928.

[2] *Roux,* 1895, No. 19.

[3] *Beams, Weed* and *Pickels,* 1933.

[4] *E. N. Harvey,* 1934; *Pickels,* 1936.

[5] *McKeen Cattell,* 1936.

[6] *O. Hertwig,* 1893, and many others. Further literature in *Morgan,* 1927, chapter 21.

for instance, the growth process — is studied in different temperatures and found to follow a similar law, this would suggest that some fundamental processes involved are chemical in nature.[1] In order to study the effects of *temperature gradients* on development, devices have been constructed in which an egg can be kept cool on one side and warm on the other; many of these experiments were done on eggs of amphibia which measure only about two millimeters in diameter.[2]

Chemical environment.

The action of the *chemical milieu* is clearly divided into two phases, one preceding and the other following the beginning of the intake of food. Most of our information concerning the former phase has been gathered from studies of marine eggs whose dependence on the composition of the ambient medium, the sea water, is particularly close.[3] The salts dissolved in the sea water are significant in a double way: first, *osmotically*, in proportion to their total concentration, and second, by the *specific* effects of different ions on protoplasm. The osmotic pressure of the sea water can be lowered or raised by diluting with distilled water or by evaporation. The methods to study the individual component ions in their relative importance are, however, more laborious. Chemical analyses of sea water have revealed the formula of its composition. On the basis of this formula artificial sea water can be made up. A single salt whose effect on development is to be tested, is, then, simply either omitted entirely from the mixture or added in varying concentrations; however, care must be taken to compensate for the alteration of the osmotic pressure by changing the concentration of the other substances proportionately. Extensive studies of this kind have disclosed that the chemical adequacy of the milieu depends not only on the *presence* of definite elements but also upon the maintenance of a definitely *balanced ratio* among them.[1]

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[1] See *Needham*, 1931, vol. 1, part III.

[2] *Vogt*, 1927; *Huxley*, 1927; *Gilchrist*, 1928.

[3] *Herbst* (1897) has inaugurated these studies; all available data have been collected in *Needham's* monograph.

These few remarks illustrate the general procedure followed in testing the environment. Most of the physical and chemical factors thus far identified in the environment have, at one time or another, been examined in their bearing on developmental processes. Newly discovered factors are scrutinized almost as soon as they become known.[2]

Alteration of environment as experimental method.

Thus far, we have touched upon only one aspect of the effects of environment on the developing organism, namely,

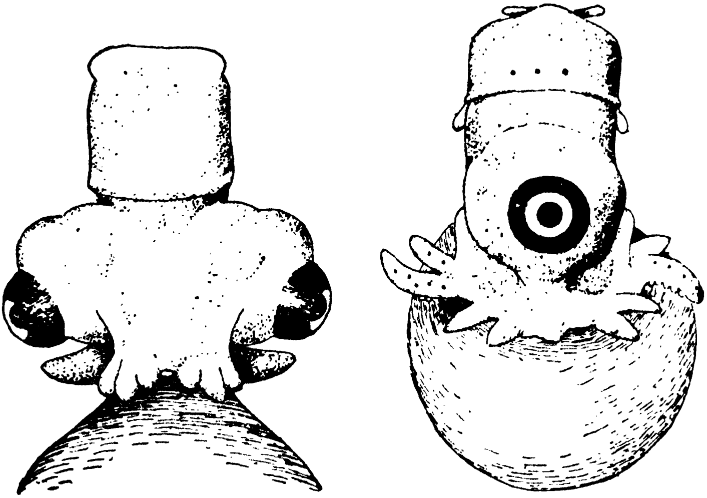

FIG. 21. Cyclopean development in the squid (*Loligo vulgaris*) following chemical treatment of the embryo. (From *Ranzi*, 1928)

A, Normal embryo on top of yolk sac, showing paired eyes and bilateral rudiments of siphon and arms.

B, Cyclopean embryo obtained by exposing the blastoderm of the egg to a solution of 1% LiCl in sea water for from 12 to 48 hours. Note fusion of the lateral eye lobes into a single median structure. × 20.

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[1] See *Heilbrunn*, 1937.

[2] Following the recent discovery of "heavy" water, experiments were started almost immediately to determine its effects on development. See Tabulae Biologicae, vol. *6*, p. 344; 1937.

that in which these effects themselves are the problem. But there is another angle: once familiar with the modifying effects of an external agent, we may use it as a means of producing at will those modifications of development which we know that it will bring about. Eggs treated with certain chemical substances (e.g., magnesium salts, ether, alcohol) often develop into *cyclopic monsters* instead of into normal individuals with paired eyes[1] (Fig. 21). The application of other chemicals (e.g., sodium chloride) can prevent the closure of the spinal cord (*spina bifida*). By omitting calcium from artificial sea water early segmentation stages of marine eggs can be caused to break up into their component blastomeres;[2] the cells which usually adhere to one another round off and separate. This is a convenient method of isolating individual blastomeres for the study of their developmental capacities.

The Study of Internal Factors

Just as the environment is composed of a great number of diverse factors which analytical investigation must endeavor to disentangle and study singly, so the developing organism itself consists of numerous components whose capacities, activities, and influences cannot be clearly discerned unless the complex situation is broken down into simpler constituents.

The relation between the whole and its parts.

The fundamental problems as outlined above (p. 109) concern (1) the relations between the whole germ and its parts, and (2) the influence of the parts upon one another. We must, therefore, devise methods by which these relations can be altered at will; experimental attack is possible only to the extent to which such methods are practicable. But inasmuch as many of them affect the integrity of the organism, one realizes that there are definite limits beyond which one cannot carry the experiments without threatening basic vital necessities; even the most cleverly contrived experiment is of no avail if the living test object does not survive it. Nevertheless, by choosing proper objects one has been able to perform all the experiments that were deemed essential.

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[1] *Stockard*, 1909; reviewed by *Adelmann*, 1936; cyclopia in cephalopods: *Ranzi*, 1928.

[2] *C. Herbst*, 1900.

We focus on a particular fraction of a developing organism and call it a *part;* but is it a part merely in the descriptive sense of parcel, or is it an *integral* part, engaged in *active interchange* of influences with other parts of the community? Only complete separation from the rest can tell. If both the separated part and the rest of the system behave as if nothing had been changed and as if they were still united, this is certainly proof of their complete autonomy and mutual independence; such parts have simply lived side by side without drawing special benefits from each other. If, on the other hand, a part shows after isolation a distinct loss or gain in capacities, we take this as an indication that some stimulating or inhibiting or otherwise controlling factors must have been impinging upon the part from the rest of the germ prior to their separation.

Since part and remaining organism are usually quite disparate in size and importance, the consequences of experimental separation are not equally serious for both. If we keep a part alive in order to study what it has lost or gained by its removal from the organism, we call this an *isolation* experiment. If, conversely, we focus our attention on the organism minus the removed part, we call this a *defect* experiment, because the elimination of one of its components has rendered the organism defective. Isolation and defect experiments, therefore, complement each other. Both aim at the interruption of all active connections between the fragments. This implies the severing of mechanical continuity and the abolishing of mutual pressure, tension, exchange of chemical substances, conduction of stimuli, and possible effects of radiation. The destructive measures of isolation and defect experiments are, finally, supplemented by a third, reconstructive, method in which the previously separated fragments are recombined in either the old or a new configuration. Experiments of this kind may be termed *recombination* experiments.

Specific and unspecific relations.

The changes from normal that a part undergoes after its isolation from the rest of the body can be roughly subdivided into two classes: *specific* and *unspecific* changes. These changes are attributable, accordingly, to the elimination or introduction — as the case may be — of two classes of factors: *specific* and *unspecific* factors. Although the distinction is not always clear-cut, one can, at least in general, tell the factors that have an immediate bearing on the *quality* and the *character* of the developmental result from others which, though being indispensable for the result, do not determine its nature. The former group comprises the specific, the latter group the unspecific factors. They have also been called [1] *determining* and *realizing* factors, respectively, because it is the *specific* factors which *determine* the character of a process, while the *unspecific* factors merely assist in its *realization*. Among the unspecific reactions we count the so-called "*trigger*" reactions; this term connotes the release through some action of a process whose character, pattern, vigor, progress, speed, and so forth, have no relation whatever to the corresponding traits of the releasing event, just as the pulling of the trigger has no relation to the course, spin, speed, and penetration of the bullet.

The isolation of parts.

The isolation of a part interrupts both unspecific and specific relations. Evidently, however, the isolation experiment does not permit us to distinguish the two classes from each other, because if a given part in isolation fails to do something which it would have done in the intact germ, this can be ascribed to lack of ability just as well as to lack of opportunity; in the former case specific factors, in the latter case unspecific factors would have dropped out. In this dilemma the recombination experiment comes to our aid. If the isolation experiment establishes the existence of relations between parts, the recombination experiment goes one step further in sorting the specific from the unspecific ones.

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[1] *W. Roux*, 1912.

The recombination of parts.

A recombination experiment consists of restoring to an isolated part conditions which it has lost through its isolation. The most logical approach to this end is to re-incorporate the part into another germ. The part is removed from its old connections and allowed to enter new ones. These may be either identical with the original ones or differ from them in a known way. Thus positive evidence regarding specific and unspecific interactions between part and germ can be gained to supplement the negative evidence of the isolation and defect experiment.

A survey of the most common techniques employed in these three types of experiments follows.

ISOLATION EXPERIMENTS

Isolation of egg fragments.

Fragments of eggs at pre-segmentation stages, either before or after fertilization can often be obtained by mechanical procedures: the eggs can be shaken to bits, or cut with an appropriate fine instrument, or constricted by means of a ligature. In view of the minute size of most of the suitable types of eggs, special methods and techniques had to be developed to allow their handling. The active protoplasmic portion of an egg measures not more than from a fraction of a millimeter to at best little more than one millimeter in diameter. Eggs consisting largely of protoplasm are, therefore, correspondingly small. The comparatively enormous size of the eggs of birds and similar forms is solely due to the abundance of stored nonliving yolk. It is obvious that for operations in the minute dimensions of the egg protoplasm the tools and techniques of ordinary dissection would be altogether too crude. Thus a special "microsurgery" has been developed in which both steel and glass instruments are in use.[1] The points of fine steel needles can be ground to form a cutting blade, but for many purposes the cutting with glass needles is preferable; in view of the soft consistency of the objects the greater resilience of glass is no serious drawback. Glass needles are drawn out in the flame from glass rods and can be fashioned by the use of microburners into short, stout, and resistant points for the purpose of pricking, or into slender and elastic points for the purpose of cutting. To manipulate eggs with the least possible damage, the use of hair-loops has been found advantageous; these are made by bending a child's hair to form a loop and sealing the ends into the capillary end of a pipette, letting the kink protrude for a little less than a millimeter. The hair combines the proper amounts of elasticity and rigidity necessary to manipulate the eggs without injuring them.

By the use of these instruments fragments can be cut from eggs under a microscope. Since the nucleus is essential for the permanent maintenance of cell life, only those fragments of eggs survive which contain the egg nucleus. Fragments devoid of nuclear material are doomed to die sooner or later but can be saved by the entrance of a sperm whose nucleus will vicariously take control of the vital functions of the fragment (see p. 183). The separation of blastomeres or of larger parts of the segmenting egg can also be achieved by mechanical means; in marine eggs it can be facilitated by the application of calcium-free sea water which, as was mentioned above, causes a loosening of the cellular contact. Fragments of older germs can be excised either with glass needles or fine iridectomy scissors.

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[1] *Spemann*, 1921, has given an account of the microsurgical procedures in use in his laboratory.

Provisions for prolonged survival of isolates.

If studies of the effects of isolation are to be carried beyond the stage of the first acute changes, provisions must be made to keep the isolated fragments alive for a sufficient length of time. Fragments of marine eggs are adequately provided for by being reared in their normal medium, sea water. Fresh water eggs, such as those of fishes or amphibia, which must be removed from their membranes at the time of the operation, are thereby deprived of protection against bacterial infection and disintegration. While formerly the neglect of this fact used to diminish the experimental returns considerably, the recent introduction of aseptic methods of operation and the use of bacteriologically sterilized media have greatly reduced the mortality of operated germs.[1] Saline solutions of an empirically tested composition were found to be a better medium than pure water for the rearing of fragments of fresh water eggs.[2]

Explantation and tissue culture.

Parts of older embryos are more particular in their requirements, and special methods had to be devised to insure the survival of such parts in complete isolation. It is obvious that fragments cannot express any of their developmental capacities unless they are in full vitality. Therefore, the success of an isolation experiment hinges on our ability to provide the isolates with those vital necessities which are under normal conditions supplied to them by the rest of the body. The completeness with which these necessities can be substituted under artificial conditions limits the length of time for which isolated pieces can survive. Just as cut flowers wither fast if they are kept dry, survive longer in water, and still longer in appropriate salt solutions, so the survival of isolated fragments of animal tissue depends upon the adequacy of the medium into which they are transferred. The method of transferring fragments from the organism into an artificial environment is called *explantation*. If the conditions are such as to guarantee to the cells prolonged viability and vital activity, one speaks of *tissue culture*.[1]

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[1] *Woerdeman*, 1930.

[2] *Holtfreter*, 1931.

Vital necessities of explants.

In order to understand the demands which these methods must satisfy to be successful, we must realize what the fragments of an embryo lose when isolated from the rest. The main conditions normally furnished by the organism and lost with isolation are: (1) protection against harmful influences of the environment; (2) oxygen supply; (3) adequate food supply; (4) proper physical and chemical surroundings. The first two points are attended to in every explantation experiment, the latter two points, however, only in the true tissue culture methods. Protection against mechanical injury is warranted by the use of proper containers, protection against bacterial disintegration by the use of aseptic methods and sterile media. Oxygenation is attained by leaving a sufficient amount of oxygen enclosed in the container and renewing the supply before exhaustion.

Techniques of tissue culture.

Under these conditions inorganic media of proper osmotic pressure and proper pH will satisfy the cells for a considerable length of time. The younger the cells, the longer this period will be, because younger stages still possess some yolk reserves as a means of subsistence. In the true tissue culture experiments, however, one embeds the cells in a nutrient medium from which they can draw food stuffs after their own reserves have become used up. As a matter of fact, the testing of various media of different composition has developed into an important method to determine what the basic requirements of cell life are.[2] Thus far, no synthetic medium has been found to satisfy the cells permanently.

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[1] For comprehensive treatises on tissue culture see *Fischer*, 1930; *Parker*, 1938; the morphological applications of the method are emphasized in *Strangeways*, 1924; *Levi*, 1934.

[2] *R. Parker*, 1938, has surveyed the subject in a monograph.

Prolonged survival is possible only provided some organic substances obtained *from the organism itself* are added to the culture medium. Body fluids such as lymph, blood serum, blood plasma, peritoneal fluid, liquor of the eye chamber, and the like, have been successfully used as media. They all contain certain unknown factors, which means that we have not yet reached the point where we could culture isolated cells in ideally constant and absolutely controllable conditions.

The nutrient medium in which an explanted fragment is embedded is changed at intervals and replaced by fresh medium. At each transfer the fragments are washed so as to remove the accumulated waste products of metabolism.

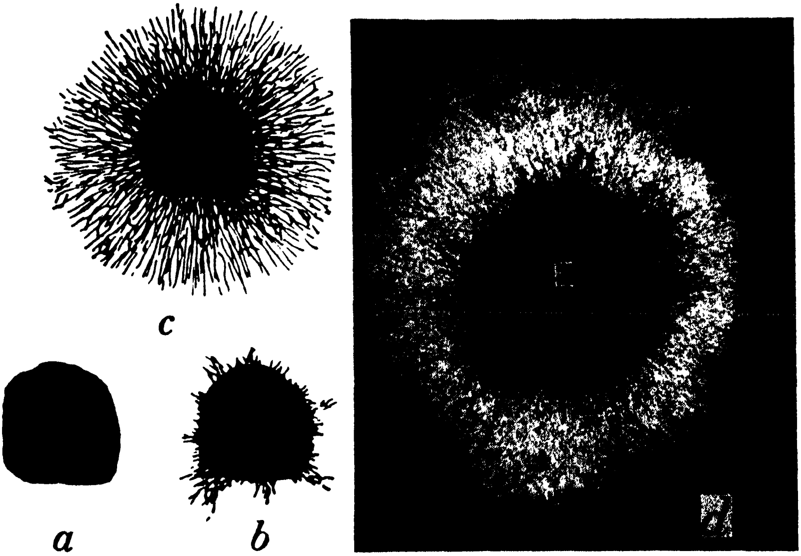

Fig. 22. Tissue culture of embryonic chick fibrocytes (mesenchyme), cultivated in coagulated blood plasma.

a, immediately after explantation; *b*, on the first day; *c*, on the second day; *d*, on the third day of cultivation.

From the border of the explanted fragment, large masses of cells migrate into the surrounding medium forming a dense veil of new tissue around the original fragment.

E, Old explanted piece; G, Fringe of newly outgrown cells; M, Medium. × 15.

Under these conditions, guaranteeing protection, oxygen, constantly replenished nutriment, and waste removal in place of the lacking excretion mechanism, fragments of the embryo can be kept alive and active for long periods of time. The cells can grow, divide and differentiate (Fig. 22). Functional activity may develop or, if present at the time of explantation, continue. In the case of many types of cells and tissues, however, additional attention must be given to the *physical properties* of the medium. Some cells can grow and move only in a medium of particular consistency; they require a fibrous framework as a substratum.[1] Several of the organic media mentioned above coagulate when they are removed from the organism, and these coagula with their spongy consistency present excellent substrata.

A major advantage of the tissue culture method lies in the fact that the living fragments raised in isolation can be kept under continuous direct observation so that processes which otherwise go on concealed in the interior of the embryo become optically accessible and can be watched under the microscope. A selection of topics advantageously studied by the method of tissue culture includes mitotic cell division, particularly the behavior of the chromosomes and the spindle; phagocytosis; migration of cells; aggregation of cells; tissue formation; production of pigments; differentiation of fibers; reaction of cells to stimuli, heat, drugs, radiations, injury, infection; and the behavior of tumor cells.[2] The standard technique employs a cover slip on which the coagulated drop of medium containing the culture rests and which is then sealed to a hollow-ground glass slide whose depression forms a small air-containing dome over the culture (Fig. 23). Other methods using glass tubes or small glass flasks as containers are also in use. Moreover, a number of apparatus have been described which make it possible to per-fuse a culture with a stream of steadily renewed medium[1] instead of keeping the medium stagnant as in the usual procedure.

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[1] *Harrison*, 1914.

[2] The files of the international Journal, Archiv für Experimentelle Zellforschung, contain the major part of the literature on tissue culture, its techniques and applications to biological problems.

Besides being the superior method of studying parts of the organism in isolation, tissue culture has the additional advantage of providing us with a constant supply of controlled strains of living cells[2] which serve as a standardized material for the testing of the factors involved in cell metabolism, growth, differentiation, regeneration, etc. (see examples 89, 93, 114, and 116 to 118).

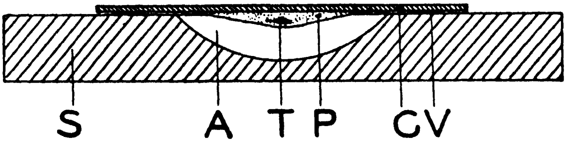

FIG. 23. Tissue culture in the "hanging drop." Longitudinal section. A drop of blood plasma (P), spread on a glass cover slip (C), receives the tissue fragment to be cultivated (T), and is then coagulated by the addition of a drop of cell-free embryo extract. The culture is covered with a hollow ground glass slide (S), inverted and finally sealed with vaseline (V) and paraffin. The enclosed air space (A) provides the tissue with oxygen for a few days. This set-up permits continuous microscopical observation.

Interplantation.

Instead of embedding fragments of tissue in extraneous media prepared from body fluids, one sometimes chooses the converse way and introduces the pieces directly into the fluids or other neutral surroundings of a partially or wholly developed organism. This method has been called *interplantation.*[3] Fragments of developing eggs or embryos of amphibians have been reared in sockets of enucleated eyes of older individuals.[4] Lymph spaces under the skin and the peritoneal cavity have likewise been used with success.[1] In the chick a very satisfactory method of interplantation has been worked out which consists of depositing the fragments of blastoderm to be cultivated on the chorio-allantoic membrane of an older egg[2] (Fig. 24). The membrane reacts to the local irritation and surrounds the implant with a richly vascularized tunic of indifferent tissue offering excellent conditions of survival, growth and differentiation, while the great distance and the separation between the graft and the nursing host embryo preclude the incorporated fragment from being reached by any but the most diffuse and general influences. Owing to the ubiquitous presence of some chemical emanations from the host body, interplantation does not offer conditions of pure isolation; but neither does the true tissue culture method in which substances gained from an organism are employed as media.

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[1] *De Haan*, 1928; *Carrel* and *Lindbergh*, 1938.

[2] So-called pure cultures; see *A. Fischer*, 1930. When a composite tissue containing a mixture of cell types is explanted, the various components often segregate by virtue of their different reaction to the changed conditions. Some grow faster than the rest and finally can be isolated in pure form (Fig. 104). Sometimes the more susceptible components of the tissue succumb, leaving only the most resistant cell type alive for continued cultivation.

[3] *Dürken*, 1926.

[4] *Kusche*, 1929.

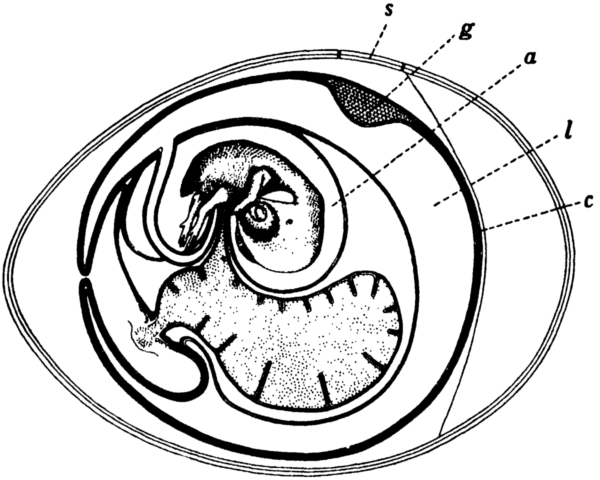

FIG. 24. Method of chorio-allantoic grafting. (From *Willier;* original) Host embryo with graft incorporated into the chorio-allantoic membrane. *a*, Amniotic cavity. *l*, Allantoic cavity. *c*, Chorio-allantoic membrane. *g*, Graft embedded in the mesenchyme of the membrane and vascularized by its blood vessels. *s*, Window in the shell through which the graft has been introduced.

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[1] *Holtfreter*, 1929; *Bautzmann*, 1929.

[2] *Danchakoff*, 1924; *Hoadley*, 1926; *Willier*, 1927.

DEFECT EXPERIMENTS

The chief difficulty of isolation experiments, namely, to secure conditions under which isolated fragments can survive, is usually absent in *defect experiments;* for, the defective body left after the removal of one part or other still represents the major portion of the organism and is, therefore, very likely to continue its basic vital functions. Inasmuch as in a defect experiment the fate of the removed part is of no special interest, defects may be produced simply by destroying the part in question. Several methods are available to this end.

Removal of germinal vesicle.

The egg *nucleus* (germinal vesicle) can be destroyed by the application of X-rays or radium radiation.[1] If the proper dose is administered, the nucleus succumbs owing to its greater susceptibility, while the cytoplasm retains its vitality and can develop after being fertilized by a normal sperm cell. Similarly, a proper dose of radiation kills the nucleus of a sperm cell without abolishing its agility and fertilizing power. The nucleus of the amphibian egg has been successfully removed by suction[2]: a micropipette is used to suck up a small area of superficial protoplasm in which the nucleus is contained.

Local injury to cytoplasm.

Cytoplasmic defects are generally obtained by mechanical means. If the problem is to prevent a certain area or definite local materials of an egg from taking part in further development, local pricking with a fine glass needle often yields the desired effect. In many eggs, especially if there is an excess of calcium ions in the medium, only a small amount of substance flows out at the point of puncture, coagulates rapidly and thus automatically stops further flow. In other cases, however, the method cannot be applied because more delicate eggs burst when pricked. As in most biological experiments, success here is largely conditional upon the finding of suitable objects that will stand the intended treatment.

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[1] *G. Hertwig,* 1920.

[2] *Curry,* 1931.

Defect experiments sometimes produce entirely different results depending on whether the killed part has been removed or left in place. If mere destruction without removal is intended, *heat* is a convenient means of achieving it; one uses needles heated by electricity (thermocauters).[1] A very elegant method to kill sharply circumscribed parts of eggs or early embryos is based on the lethal effect of *ultraviolet* light.[2] The short-wave radiation of a mercury arc lamp of sufficient intensity kills living protoplasm after a certain length of exposure.[3] If a beam of such light is narrowed down to fractions of a millimeter in diameter and is then directed against a given spot on the surface of an egg or against a given blastomere of a segmentation stage, it eliminates the affected portions from further development while the shaded portion of the germ remains intact.[4]

Differential susceptibility.

While in the cases just mentioned localized defects are obtained by *local administration* of the destructive agent, there is frequently another way open to arrive at the same end. That peculiar property of organisms called *differential susceptibility* (see later, p. 375) permits us to apply injurious agents *diffusely* and still produce *localized* defects. From its very earliest stages the organism is not a homogeneous system. There are always some parts that are more sensitive than others; that is to say, the same agent, whether radiation, heat, shock, or drugs, affects some parts more strongly than others. We can make use of this differential susceptibility in order to produce localized effects. We simply apply an agent of known harmful effect in such dosage as will kill the more delicate parts, while remaining below the threshold of harmfulness for the more resistant parts. The differential effects of radiations on the egg nucleus mentioned above serve as an example. During later stages of development chemical agents are commonly used for the purpose. The embryo is immersed in a chemical solution (salts, acids, anæsthetics, dyestuffs, etc.) of known toxicity.[1] After having been exposed to these agents for a proper length of time, the germ or embryo shows localized defects in the regions of greatest susceptibility which have succumbed in the treatment. Returned to normal environment, the rest of the germ may continue to develop. It is obvious, however, that this method leaves the decision as to where a defect is to occur with the germ rather than with the experimenter.

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[1] *Hegner*, 1911; *Reith*, 1926; *Seidel*, 1929.

[2] *Schleip*, 1923; *Hinrichs*, 1927.

[3] *Tschachotin*, 1912.

[4] *Seidel*, 1932.

RECOMBINATION EXPERIMENTS

Transplantation.

Recombination experiments confront us with the task of reconnecting an isolated fragment with an organism in such a manner that not only spatial and mechanical union but opportunity for veritable interaction and communication is re-established. The living fragment must be incorporated, not merely stuck on, to a living organism. The methods used to this end are generally called *transplantations*.[2] Transplantation, originally used only in horticulture and surgery, has of late become one of the most important tools in the study of developmental relationships. Transplantation involves the exchange of different parts within the same individual or from one individual to another. The former type is called *autoplastic;* the latter is designated as *homoplastic,* if the two individuals belong to the same species; as *heteroplastic,* if they belong to closely related species; and, as *xenoplastic* if they belong to widely distant species. The individual from which the fragment is taken is called the

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[1] *Cotronei*, 1921; *Child*, 1928; *Ranzi*, 1928.

[2] For a detailed description of the following examples of transplantation, as well as for references to the original literature, see the comprehensive treatise of *Korschelt*, 1931.

donor, while the individual which is to receive it is called *host.* The location into which the graft is placed may be either the same as the one from which it has been removed (*homotopic* transplantation) or a different one (*heterotopic* transplantation). Finally, a graft, instead of being exchanged for some other part, may be added as a supernumerary part to a complete organism, i.e., one from which nothing has been taken away (*implantation*).

The range of transplantability.

Far from being applicable universally and indiscriminately, transplantation is strictly limited in its possibilities by the constitutions of donor and host. On the whole, the chances for successful transplantation decrease with increasing height on the evolutionary scale of the employed species, with increasing age of donor and host, with increasing remoteness of family or species relationship between donor and host, and, finally, with increasing size of the transplants.

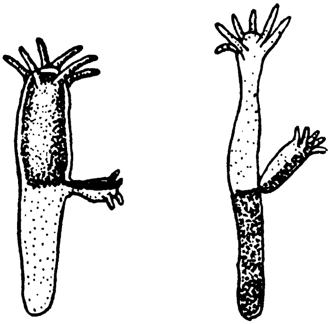

FIG. 25. Graft combinations of uncolored and colored specimens of the hydroid, *Chlorohydra viridissima.* (After *Goetsch* from *Korschelt*) The coloration of the latter is due to infection of the tissues with symbiotic algae. The lateral buds seen in the picture have developed in the border zone after the fusion. Their coloration indicates that building material has been contributed by both components.

In coelenterates, such as hydroids, and in lower worms, entire heads and fair-sized portions of the body can be successfully transplanted to other individuals of the same or of another species in the fully developed stage (Fig. 25). The same is true for certain annelid worms; in the earthworm, for instance, to mention just one example, a head or a tail with numerous segments attached, when grafted laterally to the body wall of another individual, will heal in and yield a worm with three ends (Fig. 26). Obviously, the success is linked with the well-known capacity of fragments of severed earthworms to survive long enough to permit the gradual regeneration of the lost portions. Thus, a grafted piece can likewise maintain its own life pending the establishment of an intimate fusion with the host's body. In the adult stage of higher animals, such a feat would be impossible for the reason that the grafted parts would die long before the indispensable new ties with the host would have been established. On the other hand, even in higher forms major portions of the body can be transplanted if the operation is performed during the embryonic or larval period. Pupae of insects, for instance, have been divided and recombined, anterior with posterior portions in tandem; imagos hatching from these turned out to have composite bodies (Fig. 27). Similar compositions were effected by grafting together head and tail parts of amphibian embryos (Fig. 28). In both the insect and amphibian experiment, *chimeras* could be produced by fusing parts of individuals belonging to different species.

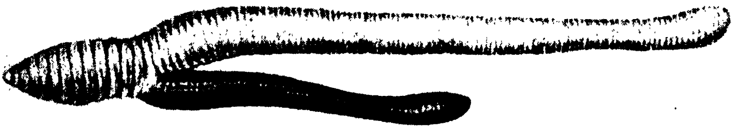

Fig. 26. Permanent union by transplantation of two individuals of different species of earthworms. (From *Korschelt*, 1931) A posterior fragment of *Eisenia foetida* (dark) has been grafted to the side of an *Helodrilus longus*. Photographed 97 days after transplantation.

In post-embryonic stages of vertebrates, it is no longer possible to graft large pieces of the body, but the transplantation of individual organs of smaller size is still possible, sometimes only earlier in life, sometimes even in the adult stage. Difficulties accumulate as the animals grow older, and in forms in which a larval period is interpolated between

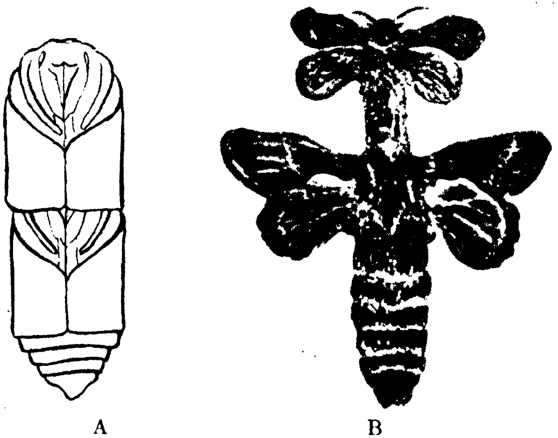

Fig. 27. Metamorphosis of graft combinations of pupae. (After *Crampton* from *Korschelt*)

A, tandem fusion of two pupae after resection of the rear end of the anterior partner and the front end of the posterior partner.

B, metamorphosed tandem combination of *Cecropia promethea* and *Samia cecropia.*

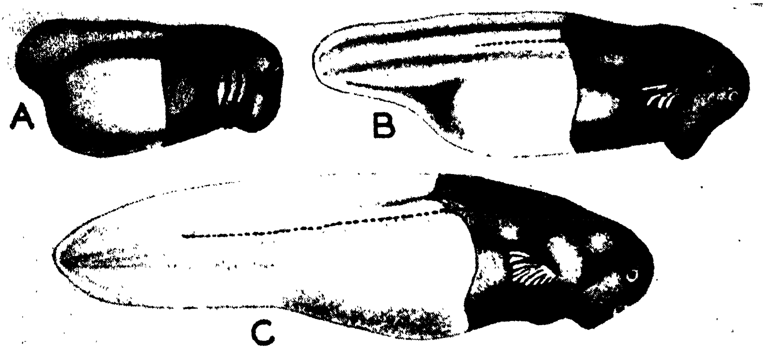

Fig. 28. Graft combinations of frog larvae of different species. (After *Harrison* from *Korschelt*) Anterior half of an embryo of *Rana sylvatica* grafted to the posterior half of an embryo of *Rana palustris.* Note the dark lateral line sense organs growing tailward from the anterior part. × 10.

A, 2 hours after the operation.

B, 26 hours after the operation.

C, 51 hours after the operation.

the embryonic and the adult phases, chances for successful transplantation drop markedly with the onset of metamorphosis. While in larval individuals of amphibians, complex organs, such as limbs (Fig. 122), eyes, and inner organs, can be made to heal and resume normal function, adult forms of the same species are frequently found to be refractory to these operations although they will still tolerate the transplantation of smaller or simpler fragments, such as muscle, skin, bone, spleen, heart, or glands. The minimum of transplantability is reached in metamorphosed arthropods and higher vertebrates, particularly mammals. Although tails of rats have been transplanted under the skin and surgeons report several cases of transplantation of entire joints in men, success is generally limited to the transplantation of small organ fragments or tissue fragments, such as flaps of skin, fragments of bone, stumps of tendons, pads of fat tissue, segments of blood vessels, and the like.[1]

Perishing transplants.

If we speak of successful transplantation, we have in mind permanent incorporation of the transplanted piece. Not infrequently transplants which have survived for a certain length of time, nevertheless perish in the end and are gradually replaced by some of the host's own tissues. Thus, transplanted skin covering a wound is often supplanted by host cells; it, therefore, acts merely as a temporary scaffolding upon which the host may regenerate new skin, rather than as a permanent living contribution to the body. Substitution by host tissue is also a common fate of transplanted bone, glands and muscles, with connective tissue playing the invader. Such happenings, of course, interfere seriously with the purpose of transplantation, which is to test the capacities of the transplanted fragments; unless the transplants can be made to persist in full vitality and, if possible, functional capacity, the experiment is a failure.

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[1] Occasionally a larger organ (kidney, leg) can be made to heal in, provided its blood supply is restored immediately after the transplantation (*Carrel*).

The biochemical differential of individuals, species, etc.

Although technical skill can go very far toward improving transplantability, it is powerless to do anything about the natural resistance to transplantation arising from *chemical incompatibility between graft and host.* Each individual, each species, each genus, and so on, has its characteristic biochemical differential which is ingrained in the substance of every cell. There is a chemical tag attached to the cells identifying them as belonging to a given individual, family, species, genus, order, class, and phylum.[1] Whether these identification marks consist each of a discrete chemical compound or whether they express merely different groupings of the same compounds, we do not know. We do know, however, that they follow a definite order in that they diverge and become increasingly dissimilar, as we move up along the scale from individual to class; individuals of the same species have more in common in their basic biochemical constitution than do individuals belonging to different species. Again, all species belonging to the same genus exhibit greater biochemical similarity than is found among different genera; and so forth. The biochemical differences among individuals are called "individual differentials."[2] They follow a definite rule, to which the genetic relationship is the key. *Individual differentials* increase according as the degree of consanguinity decreases; they are least marked among brothers and sisters, more pronounced among cousins, still greater among distant kin, and greatest between unrelated members of the species or different races. *Species differential* is that part of the chemical constitution of an individual which is in common to all members of the species while being discriminative of the particular species as against other species. Again, it is greater between more distantly related than among more closely related species.

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[1] Signs of this biochemical specificity can be detected by serological tests.

[2] *L. Loeb*, 1920.

Transplantability and biochemical differential.

Now, from the extensive experiences with successful and futile grafting a rule has crystallized stating that *transplantability goes parallel to genetic or phylogenetic relationship;* that is, the more closely host and donor are related, as species and as individuals, the better are the prospects for transplantation. Transplantations succeed best when performed between sisters and brothers, and the chances for success decrease in the following order: parent to child, child to parent, cousins, distant relatives, nonrelated members of the same species, members of different species, members of different genera, members of different orders. Since this is also the order in which the biochemical "individual differentials" diverge, it follows that *transplantability is roughly in inverse proportion to the biochemical differential between donor and host.*[1] Of course, in a given case transplantability is an all-or-none proposition; the graft either takes or not. It may seem odd, therefore, to speak of transplantability as if it were a measurable quantity. Yet, if one operates with large numbers of cases the statistical treatment based on the varying ratio of successful to unsuccessful cases produces quantitative data.

An evaluation of the known facts leads to the following conception: (1) A limited amount of biochemical strangeness between graft and host can be tolerated without endangering their successful union. Within these fixed limits host and donor tissues are to all practical purposes compatible. (2) If the strangeness exceeds this tolerable limit, the graft fails to take.

If we perform transplantations between a large group of individuals whose biochemical differentials lie close to the limit of mutual compatibility, the result will not be uniform. Random fluctuations will shift the critical mark for different individuals, and some will be successes, others failures. But it is evident that the *proportion* of successful to unsuccessful cases will steadily increase, the more the biochemical dissimilarity between donor and host exceeds the range of absolute tolerance.

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[1] *L. Loeb*, 1921. — *Schultz*, 1918, has pointed out that there is also an approximate parallelism between the transplantability of tissues from one species to another and the capacity of hybridization among these species.

In lower forms, in which the biochemical specialization is less pronounced than in higher ones, the range of transplantability is correspondingly wider. While in amphibians, for instance, even adult tissues can sometimes be transplanted between members of different species, grafting in mammals is definitely confined to members of the same species, and often even more rigorously to individuals of related or identical parentage. In man the biochemical specialization seems to have become so high that the prospect for interindividual (homoplastic) transplantations is very unfavorable and that satisfactory returns can be expected only from autoplastic transplantations.

The fate of unsuccessful grafts.

Having established the parallelism between transplantability and biochemical relationship, one may ask next for the reasons why biochemical incongruity between tissues should preclude their union by grafting. Two possible explanations come to our mind. Either the chemically different environment and nutriment of the host disagree with the grafted tissue so that it perishes from insufficiency, or it is the host which is refractory to the union and throws off or otherwise destroys the graft. Do the grafts quit or are they ousted? Experimental evidence indicates that they are eliminated by actions of the body: A piece of rat tissue which would perish when transplanted into a living chick, survives undamaged if explanted into a medium consisting of the body fluids of the chick.[1] So far as the graft is concerned, the chemical milieu is as strange in one case as it is in the other; if anything, one would expect the explant to be at a disadvantage. But since it is the transplant which succumbs, one must conclude that the body rejects the graft rather than vice versa. The rejection takes the form of a mobilization of chemical and cellular reactions directed against the intruder. These reactions are similar in kind to those noted in bacterial infection. Invading swarms of lymphocytes, in coöperation with specific substances capable of dissolving the strange proteins, break down the foreign tissue. In favorable cases the reaction of the body against a strange transplant may remain confined to the erection of a wall of connective tissue encasing the graft and insulating it thus from the host's tissues. In most cases, however, the strange tissue is destroyed or extruded from the body.

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[1] *Carrel* and *Ebeling*, 1928.

Attempts have been made to better the chances of transplants by either lowering the resistance of the host body or reducing the biochemical strangeness of the transplants. Positive results were reported in both cases but only within very narrow limits. A weakening of the violence of the host's reaction can be obtained as a result of a general weakening of the vitality of the organism, for instance, by keeping an animal on a substandard level of nutrition.[1] It has also been claimed that allowing strange tissues to dwell for some time before transplantation in a tissue culture medium prepared from the body fluids of the prospective hosts has a beneficial effect, as if the fragments thereby acquired a biochemical disguise concealing to the host their actual strangeness; [2] but the experimental evidence for this is still far from convincing.

Prerequisites for success in grafting.

To sum up, prerequisite for successful transplantation is (1), a definite degree of genetic or systematic relationship between donor and host, the limits of which vary according to age and species employed, and (2), the securing for the transplants of conditions of protection, oxygen supply and nutrition at least as favorable as those in tissue culture (see

[1] *Morpurgo*, 1927.

[2] *Gassul*, 1923; *Rh. Erdmann*, 1927.

above, p. 153). In most cases, however, transplantation can do far better than tissue culture, mainly because of the reconnection of the transplant with a functional blood, lymph, and nervous system.

Embryonic transplantation.

The method of transplantation has been of utmost usefulness in the field of embryology. As can be deduced from the preceding discussion, the younger the stage in which one transplants, the better the prospect. From the biological viewpoint early embryonic stages furnish, therefore, exquisite material for transplantation experiments. In these stages the species differentials and individual differentials have not yet made their appearance, or, at least, have not yet been developed to the point where they could become troublesome.[1] Consequently, embryonic transplantations are feasible between animals as distant as anuran and urodele amphibians,[2] and even between amphibians and fishes. From the technical viewpoint, however, the use of younger stages increases rather the difficulties on account of the small size of the objects. Considering that the diameter of the sea urchin egg is less than one tenth of a millimeter, one can realize how difficult it is to shift individual blastomeres around, transplant them to predetermined positions, and recombine them at will in various combinations. Nevertheless, in spite of the great delicacy of the operation involved these very feats have been accomplished (see p. 272 f.). In the amphibian egg, fragments measuring only a few hundredths of a square millimeter have been transplanted from one germ to another and healed in their new position (Fig. 61). The operations are performed under the binocular microscope with the fine glass and steel instruments described above.

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[1] *Braus*, 1906b, has shown, for instance, that tadpoles of different anurans do not yet show the specific serological reactions characteristic of metamorphosed animals.

[2] *Geinitz*, 1925, and many others since. See page 363.

Fusion of whole organisms.

In connection with the transplantation methods we may briefly mention experiments aiming at the intimate fusion of two whole individuals. Undeveloped eggs of marine animals can sometimes be made to fuse with each other by crowding.[1] Two whole amphibian eggs can be united in the two-cell stage by stretching them until they assume the shape of dumbbells and then placing one across the other[2] (Fig. 58). Older embryos can be connected by slashing the body walls of the two prospective partners and fusing them along the wounds. This procedure is called *parabiosis.*[3] If healing takes place the effects of one partner upon the other can be studied. Parabiosis has been executed also in adult individuals, and success has been attained even in mammals.

MODEL EXPERIMENTS

Utility of models.

The experimental methods discussed so far have all in common that they approach the living object in a direct way. They attempt to analyze the developmental processes by interfering with the developing system directly. However, there is another course open, namely, the study of analogies and models. This course is particularly indicated in those instances in which the difficulties of a direct approach are prohibitive. Models are reproductions of natural objects or phenomena in their essential features, and an analogy is, in a sense, nothing but a model taken over ready-made from some other and better known province of nature. Physicists, in their studies of optical and electrical phenomena, have widely exploited mechanical analogies, in the former case of "waves" in stagnant fluids, and in the latter case of "currents" in moving fluids. Guided by these analogies, models could be built which permitted to treat on the familiar ground of mechanics, quasi in effigy, the problems arising in the less familiar and less readily accessible fields of optics and electrics.

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[1] *Bierens de Haan*, 1913.

[2] *Mangold* and *Seidel*, 1927.

[3] *Burns*, 1925; and others.

The consensus is that models can be very helpful guides to the solution of knotty problems, but at times can also be dangerously misleading. It all depends on how genuinely the model represents its prototype. Current physiology has many models in its employ to illustrate the activity of cells, especially in their physico-chemical aspects. There are models of resorption, excretion, cell division, nerve conduction, and muscular contraction, to mention only a few. What commends many of them is not external and superficial resemblance but the consistent inner agreement between their essential properties and those of their prototypes.

In embryology one has only sporadically resorted to the use of models,[1] and rarely has their application been carried to the point where they would have really clarified the phenomena which they were meant to represent. They were introduced to serve mostly as illustrations rather than as explanations; but even so, they have rendered some good services by suggesting possible ways of interpretation.

Example 8.[2]

Model of gastrulation.

The spherical blastula of the sea urchin egg changes into the cup-shaped gastrula by the invagination of the vegetative bottom into the animal roof (Fig. 43, i–l). This is achieved by a reversal of the curvature of the vegetative cell plate from convex to concave. Although the mechanism of this transformation is unknown, a model has been contrived which reproduces the essential features of gastrulation with some faithfulness and which might, therefore, be considered as pertinent. It is based on the fact that the colloidal cell plate has certain physical properties in common with colloids in general, for instance, with gelatine. Among these properties is the capacity to swell

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[1] *Rhumbler*, 1914, has devised numerous useful models of morphogenetic phenomena.

[2] *Spek*, 1931.

in water (see p. 32). The rate and amount of swelling of a gelatine plate depends, however, among other things, upon the concentration of the gelatine. If, therefore, a double plate of gelatine composed of two layers of different concentrations is immersed in water, the layer with the higher concentration and, hence, higher swelling capacity, expands more rapidly than the other layer, and the result is a bending of the plate (Fig. 29 A). If the original plate has formed the segment of a sphere (Fig. 29 B), corresponding to the vegetative portion of a blastula, and if the layer of greater swelling tendency lies on the inner side, a real reversal of the curvature of the whole plate is obtained, just as in the case of gastrulation (Fig. 29 C).

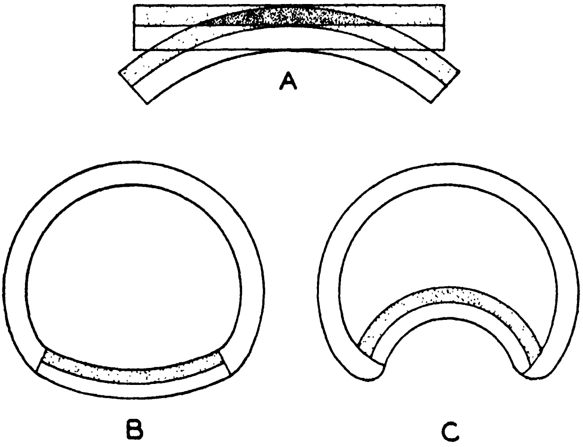

Fig. 29. Gelatine model of gastrulation. (After *Spek.*)
A, Warping of gelatine plate composed of two layers of different swelling capacity when immersed in water.
B, Model of blastula with double floor consisting of layers of different swelling capacity.
C, Same model, after immersion in water. Floor invaginating into interior.

The model suggests differential swelling of cells as the mechanism of gastrulation. It is evident that if swelling occurs at all and if it proceeds more rapidly in those portions of the cells facing the blastocœle than in the portions facing the outside medium, the observed invagination must take place. The differential swelling which, in the case of cells, could hardly be a matter of different densities, as it is in the gelatine model, might be accounted for by the different composition of the media to either side (blastocœlic fluid on the inner side, sea water on the outer side); or by a different constitution of the cells near their inner and outer surfaces.

These are concrete suggestions which can be subject to experimental tests and possibly direct verification on the living object. Pending such verification, the model remains what every hypothesis is bound to remain before it has been confirmed: a conceivable possibility and a guide to crucial experiments.

Rhythmic structures in organisms.

Another example may illustrate a somewhat different class of models. It concerns the element of rhythmicity so common in many developmental patterns. Numerous structures of the body are repetitive and appear in more or less regular spacing. The stripes of the zebra, the bands in the feathers of a pheasant, the palmar ridges (dermatoglyphics), the lamination of brain and bone, and the concentric rings forming the "eyes" on the wings of butterflies are but a few out of countless examples. In many cases, if not in most, the *spatial* rhythm is the result of *time* periodicity. That is, if the agents which produce a given structure act in periodic spells instead of continuously and steadily, the resulting pattern must necessarily be rhythmic in character. Tree rings occasioned by the seasonal cycles of growth rates are an example of this class. However, there are other rhythmic structures which do not seem amenable to this kind of interpretation; e.g., striped skin patterns or the segmentation of the body of a ringworm; nowhere in their production do we find periodical agents. The substratum in which they arise shows no rhythmicity, nor do the coloring or segmenting agents seem to "beat" rhythmically. Yet the results turn out to be rhythmic. In this puzzling situation, the observation of rhythmic precipitations in inorganic chemical systems seems to be of special interest.

Example 9.[1]

A model of rhythmic patterns.

A plate of gelatin is soaked in potassium bichromate. A drop of silver nitrate is then placed on the moist plate. The silver solution slowly diffuses into the gelatin and there reacts with the bichromate leading to the precipitation of dark silver chromate. This precipitation, however, instead of being evenly distributed, occurs in a pattern of concentric rings which are separated by distinct clear spaces (Fig. 30).

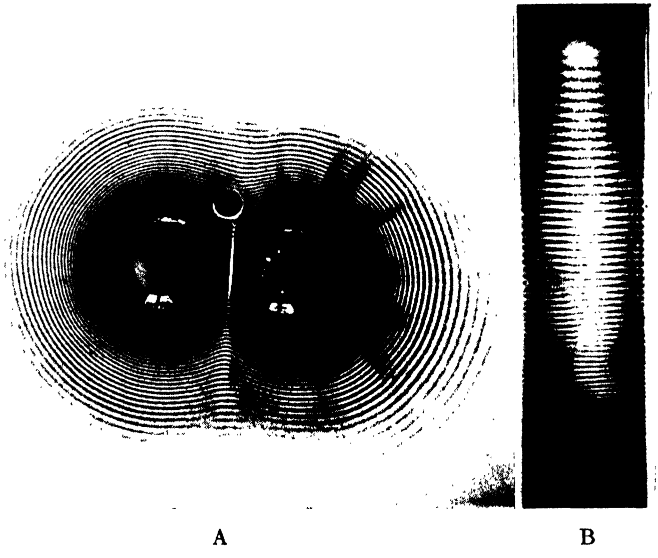

Fig. 30. *Liesegang's rings.* Rhythmic precipitations arising by slow diffusion of silver nitrate in gelatine containing potassium chromate.
A, two diffusion centers on a gelatine plate. × $\frac{5}{4}$.
B, diffusion from the bottom in a gelatine-filled tube. × 3.

This phenomenon has become known under the name of *Liesegang's rings.* A discussion of its physical basis is not within our scope. But it serves as a striking illustration of how striation can arise in a homogeneous medium even in

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[1] *Liesegang*, 1898.

the absence of periodical agents. Neither the gelatine nor the application of the silver solution contains any element of rhythmicity. And yet, the resulting pattern exhibits rhythmical discontinuity. This demonstration alone is of fundamental significance because it shows in inorganic conditions how discontinuity can arise in a continuum, and patterns emerge from chaos. The usefulness of the model, therefore, lies in that it suggests a *principle* according to which striations, stratifications, and segmentations might possibly arise. On the other hand, one must guard against taking the model too literally as if it suggested that the *mechanism* through which rhythmic structures are brought about consisted likewise merely of diffusion and precipitation in jellies. In fact, this latter contention, which once was advanced as an explanation of the rhythmic rings and bands on the wings of butterflies,[1] is clearly contradicted by a number of known facts.[2] This does not detract, however, from the merits of the model which illustrates the mode of operation rather than the nature of the operating agents, just as the wave model of the physicist illustrates the operation rather than the nature of light.[3]

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[1] *Gebhardt*, 1912.

[2] Cf. the excellent review by *Henke*, 1935.

[3] A French physician, *S. Leduc* (1910), has devised a number of interesting procedures by which certain chemical reactions in solutions are caused to precipitate membranes of such shape that they resemble the outlines of organisms. He went so far as to proclaim an inner identity between these crude imitations and their living prototypes. It goes without saying that the relation is no closer than is the resemblance between a puppet and a man. But *Leduc*'s attempt is an instructive example of the danger that lies in confounding schemes with reality.

PART THREE

PRINCIPLES OF DEVELOPMENT

THE INITIATION OF DEVELOPMENT

Basic Conditions of Development

Egg and sperm.

As a matter of convention we start our consideration of development at the moment when the egg becomes detached from the ovary of the maternal organism and begins an existence of its own. The changes of the egg cell preparatory to this stage (growth of cytoplasm and nucleus, storing of yolk, differentiation of accessory cells, etc.) may be considered as merely the overture to the play to come. The overture is followed by an intermission during which the egg is in a comparatively quiescent stage, ready to develop, but requiring some additional impulse in order to be started off. In this stage we may consider *the egg* as *the primordium of the future organism.*

This statement needs some qualification. Why, for instance, should the *sperm* be ignored in this connection? It is the general procedure in the sexually reproducing forms that the new individual arises from the fusion of a maternal and a paternal sex cell. The studies of genetics have demonstrated that with regard to inheritable details the father's contribution to the child is as significant as that of the mother. It is commonly agreed that such inheritable characters are controlled by the action of elementary units, called genes, which reside in the chromosomes. With regard to these factors, according to the incontestable evidence of genetics, maternal and paternal nuclei are essentially equivalent. Yet, if we search for the *indispensable* factors of development, we can ignore the sperm. The reason is simply that eggs can develop in the absence of sperm, whereas a sperm cell cannot develop without an egg.

Parthenogenesis.

The development of an egg without insemination is known as *parthenogenesis.* It occurs spontaneously in many arthropods and other groups of animals under natural conditions.[1] Moreover, it can be elicited artificially in many animals in which normally it does not occur.

Example 10.

Artificial parthenogenesis was first produced by subjecting the eggs of silkworms to mechanical friction or to treatment with sulfuric acid.[2] The favorite objects for these studies, however, were found among the echinoderms and vertebrates. A wide variety of physical and chemical agents can replace insemination in one respect — in inducing the egg to develop. Some of the agents producing this effect are: hypertonic solutions of salts or sugars, fatty acids, and fat solvents (alcohol, benzene, ether). Sea urchin eggs, treated for some time either with one of these agents alone or with a combination of them and then returned to normal sea water, begin to develop and produce larvae.[3] In the frog the mere puncture of the egg with a needle was found to suffice to start development,[4] especially if some blood or lymph was permitted to penetrate through the puncture holes into the interior of the egg.[5] Frog eggs activated in this fashion develop into embryos and larvae and have even been raised beyond metamorphosis.[6] Such animals are typical in all major structural and functional aspects. Their viability is reduced, but neither this effect nor the chromosomal aberrations observed in the cells can obscure the importance of the fact that typical development has taken place without the intervention of sperm.

These results prove that *the assistance of the sperm can be dispensed with in the fundamental play of developmental forces.*

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[1] *Winkler,* 1920.

[2] *Tichomiroff,* 1886; a concise review of the history of artificial parthenogenesis can be found in chapter 23 of *Morgan,* 1927.

[3] The most concerted study of artificial parthenogenesis was undertaken by *Jacques Loeb;* summarized in *Loeb,* 1913.

[4] *Bataillon,* 1911.

[5] *Voss,* 1923.

[6] *Loeb,* 1918; *Parmenter,* 1920.

In view of the wide variety of forms in which parthenogenesis has been observed, this generalization can be made without hesitation. Since the egg is capable of developing alone, the sperm must be considered as an accessory rather than an indispensable complement of development. In ordinary bisexual development, the sperm serves two principal functions.

The double role of fertilization.

First, it *activates* the egg; that is, it causes the egg to pass from a stage of inertia and readiness into one of manifest activity. It releases processes which have been waiting for realization. From the fact that such unspecific artificial parthenogenetic agents as were enumerated above, can replace the sperm in its activating role, it can be estimated how generalized and unspecific this activating role is. We call it a "trigger" reaction (p. 149) because the quality and character of the processes released in the egg are not determined by the particular nature of the releasing agent.

The second function of the sperm is to contribute the paternal *genetic factors* to the new individual. This genetic function of the sperm is more specific than the activating function inasmuch as the genes which it introduces actually affect the development of the organism. It is, however, beyond the scope of this discussion to enlarge upon the genetic side of the problem.[1]

Egg cytoplasm without nucleus.

The advantage which the egg has over the sperm cell consists in the possession of a large body of cytoplasm. Essential lack of this cytoplasm makes a spermium unfit to develop by itself. Similarly, an egg nucleus isolated and deprived of its surrounding cytoplasm fails to develop. Enucleated cytoplasm of the egg, on the other hand, is not quite so impotent; at least, it can *segment.*

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[1] For further information, see the textbooks of Genetics.

Example 11.

When unfertilized eggs of the sea urchin are centrifuged at high speed, they elongate, and the various immiscible constituents of the egg content become segregated into strata according to their specific gravities (see Fig. 32). The relatively light nucleus is thrown into an eccentric position near the centripetal pole. Under continued centrifugation the egg elongates further until it eventually breaks apart into a light and a heavy half.[1] The former contains the nucleus, the latter consists solely of extranuclear cytoplasm. If this non-nucleated half is then exposed to some parthenogenetic agent of the kind described in example 10, *cleavage sets in, much as it does in an activated egg.*[2] This segmentation can go as far as to yield a multicellular hollow sphere resembling the blastula stage. Progress beyond this stage has never been observed. But it is remarkable and highly significant that cleavage of a mass of protoplasm can proceed even this far in the absence of any cell nucleus. The irrelevance of the nucleus for the mere occurrence of segmentation is further illustrated by experiments in which the nucleus was thrown toward one pole of the egg by centrifugation whereupon the egg, when activated by parthenogenetic agents, frequently *cleaved only in the portions farthest removed from the nucleus,* with the latter remaining inactive[3] (Fig. 31).

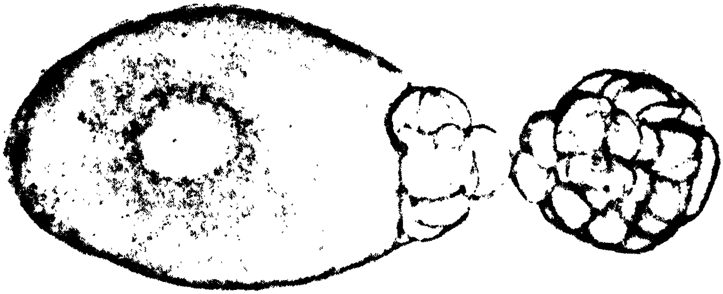

FIG. 31. Segmentation of centrifuged egg of the echinoderm, *Dendraster*. (From *A. R. Moore*) The part containing the lighter materials, including the nucleus, has remained unsegmented.

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[1] *E. B. Harvey*, 1932.

[2] *E. B. Harvey*, 1936.

[3] *A. R. Moore*, unpublished.

If development in cases like these, nevertheless, remains abortive, the blame goes, after all, to the missing nucleus rather than to the eliminated cytoplasmic portions of the cell body. For, the same enucleated egg fragments can develop further if a nucleus is restored to them. In order to obtain this effect, one allows an enucleated fragment to become fertilized by a spermium. The insemination not only activates the egg fragment and causes it to cleave, but the newly introduced sperm nucleus proves to be in all regards a competent substitute for the removed egg nucleus. It re-establishes the necessary balance and cooperation between cytoplasmic and nuclear principles upon which successful development is contingent.

Egg cytoplasm combined with a strange nucleus (*merogony*).

The procedure of introducing a sperm nucleus into a purely cytoplasmic egg fragment or into a whole egg which has been previously deprived of its nucleus is known as *merogony*.[1] The chief significance of this method lies in the fact that it permits the combination of cytoplasm of one species with a nucleus of another species of somewhat different character. As one can readily see, such a combination provides a crucial test of the relative contributions to development by the cytoplasm on one hand and the nucleus on the other. It is obvious that all characters of a merogonic organism which resemble the maternal species must have arisen on the basis of *cytoplasmic* properties, because no nuclear material of the mother has been present during the development of the merogone. Early results obtained with this method in echinoderms were suggestive but not conclusive.[2] More recent work in amphibians has produced much clearer results.

Developmental characters of cytoplasmic origin.

All experiments of this sort, however, meet with the difficulty that the viability of merogonic hybrids is very poor; in fact, the poorer, the stranger the parent species are to each other.[1] Apparently this difficulty is of a similar nature as that encountered in heteroplastic transplantations and attributable to the biochemical species differential (see p. 166). In order to be able to cooperate in the service of development, nucleus and cytoplasm must be tuned to each other, as it were. A strange nucleus in a strange cytoplasm creates disharmony. A limited degree of disharmony is compatible with development. When this critical limit is exceeded — and the stranger the two components are to each other, the earlier in development this happens — the incompatibility between nucleus and cytoplasm becomes fatal. Just how this comes about has not yet been ascertained, but there are indications that the increasing difficulty which a strange nucleus encounters in managing the transformation of yolk into cell food is at least a contributing factor.

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[1] *Boveri*, 1896.

[2] *Boveri*, 1918.

The analytical value of the study of merogonic hybrids is illustrated in the following example.

Example 12.[2]

> Owing to their precocious mortality, merogones can hardly be raised into sufficiently advanced stages of development to permit clear discrimination of maternal or paternal characters. Even among distant species, early developmental stages look too much alike. This technical difficulty has recently been circumvented by using normal germs as nurses for the feeble merogone tissues; by transplanting fragments of merogonic germs into healthy host germs the life of the former could be so prolonged that they could attain a considerable degree of differentiation. Under these conditions, distinctly *maternal features referable to the cytoplasm of the egg* were observed in the merogonic tissues, in addition to the standard genic influences transmitted through the paternal nucleus.

This is merely one demonstration that some specific prerequisites of development are present in the cytoplasm of the egg. Further examples will be set forth below. It is immaterial in the present connection whether the egg cytoplasm owes its specific properties to the fact that it is a direct descendant part of the protoplasm of the maternal body or whether it has received them through the intervention of its nucleus prior to the removal of the latter. Our present knowledge does not appear adequate to permit more than a perfunctory decision of this question. The main thing to keep in mind is that *the cytoplasm of the egg does contain specific developmental properties.*[1] Consequently, neither the nucleus alone nor the plasmatic cell body alone can be considered the primordium of a new organism; only *the egg as a whole* deserves this title.

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[1] *Baltzer*, 1920, 1922, 1930.

[2] *Hadorn*, 1937; see also *Baltzer* and *de Roche*, 1936.

The role of the environment in development.

Next, the role of the *environment* in which the egg develops calls for some comment. We know that environmental conditions affect the course of development and some pertinent examples were cited above (p. 143). But does the environment impart *specific* properties to the developing system? The fact that eggs of different species, though developing in the same environment, produce specifically different results, makes us realize that the role of the environment cannot be specific. It is an unspecific accessory. Just as the sperm, or an appropriate substitute, is indispensable for the activation of the egg, so certain influences of the environment are prerequisite for further realization of development. They can be largely identified with the basic prerequisites for life in general: adequate composition of the physical and chemical milieu (osmotic pressure, temperature, oxygen supply, sometimes light, etc.).[2]

On the whole, the conditions of the milieu affect rates rather than the character of developmental processes. However, from our discussion of differential growth (p. 36), particularly if one remembers the differential effect on morphology of an increase in growth rate (p. 46), one can appreciate the marked diversion of the developmental course which can be produced by a change in environment. For an illustration turn to example 4. Referring to the comments on page 26, we repeat that the effects of the external environment on development belong in the class of *circumstantials.* They furnish a set of conditions under which the developmental system can display its intrinsic potencies. A change in environment alters the manifestation of these potencies but not the potencies themselves, nor does the environment ever create any specific potencies. As Figure 21 has shown, activities of the environment can be responsible for the reduction or even complete suppression of an organ, but they cannot account for the specific quality of the given organ in the positive case where it appears. Occasionally, when development comes to a cross road, they may even decide which one of two alternative courses is to be followed — for instance, whether a given level of a regenerating animal is to produce a head end or a tail end (p. 377); but, again, their activities have nothing to do with the establishment and the typical organization of the head-forming or tail-forming processes between which they are allowed to choose. If we relegate the environment to the role of an unspecific accessory to development, this leaves only the egg to be considered as the seat of the *specific* instruments of development.

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[1] Concerning the role of the cytoplasm in the inheritance of certain characters, compare *East*, 1934.

[2] For a description of the effects of external factors on development, see *Jenkinson*, 1909.

Physical and chemical analysis of the egg content.

Physically, an egg consists of a body of protoplasmic matter with many inclusions, surrounded by one or several membranes, and containing a nucleus. The egg content is in the colloidal state (p. 29) — semiliquid to gelatinous; the consistency varies considerably in various species, depending largely on the amount, size, and nature of inclusions. *Chemically,* the egg consists of water to at least 50% of its volume. The solid parts, in the order of their relative proportions in dry weight, are proteins, fats, nitrogen-free organic substances (e.g., carbohydrates), and inorganic ashes. The inorganic compounds are composed mainly of the cations, potassium, sodium, magnesium, calcium, and iron, as well as the anions, sulfate, phosphate, and chloride.

This bulk analysis of the constituents of the egg is not very elucidating. It reveals the presence of essentially the same types of materials which are found in the adult organism, but tells nothing about their differential composition and distribution, or their changes during development. Extensive studies have been devoted to the chemistry of the egg and the embryo, and an enormous amount of data has been collected.[1] Sifting this material from the embryological rather than the purely biochemical viewpoint, one obtains a rather meager return. Some gross chemical changes during development were mentioned above (p. 35); many more could be enumerated. They are valuable in proving that the egg, while containing the same chemical elements as the organism, does not contain them grouped in the same combinations and assembled into the same compounds. Countless substances, for instance, many *enzymes* and *hormones*, appear only during advanced stages of development as a result of progressive synthesis from simpler constituents. Therefore, the chemical side of development without doubt involves *real emergence of new compounds* and not merely reproduction and segregation into different localities of the chemical units present in the egg. Studies of the chemical energetics of the egg have led to a deep insight into the mechanisms of energy-producing *metabolism*. Yet none of this work, invaluable as it is in its own right, so much as approaches the central problem of development, namely, that of *organization*. Chemical bulk analysis, by its very nature, fails to give account of the heterogeneous and differential distribution of the substances whose presence it reveals. As a great physiologist once put it:[1] the attempt to explore the organization of living matter with the comparatively crude methods of chemistry "is just as vain as if one tried to explore the mechanism of a watch by melting it in a crucible and analyzing the molten mass."

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[1] An impressive compilation of data on Chemical Embryology has been furnished by *Needham*, 1931.

The problem of a chemical basis of organization.

Therefore, even if the future organization of the embryo were preformed in the egg by a definite spatial grouping of different chemical stuffs, the gross methods of chemistry would not be adequate to detect such order. Pending the perfection of microchemical techniques through which one might be able in some future to test the various chemical constituents of protoplasm *in situ,* we must content ourselves with some very indirect evidence for the existence of *definite chemical organization of the egg prior to development.*

The problem of the chemical organization of the egg has two sides. The first question is: does the egg consist of an indiscriminate mixture of chemical substances, or is there a definite segregation of at least some of these substances in localized districts? The second question is: if the egg should be found to possess a definite chemical organization, does this have an immediate bearing on the organization of the embryo, and if so, to what extent?

With regard to the first question, it can safely be stated that a certain amount of chemical differentiation and segregation is present in the body of every egg. Some of it can be seen directly under the microscope, as many of the substances contained in the egg which are immiscible with each other form distinct droplets or globules suspended in the surrounding plasm; the density of these inclusions serves as a convenient indicator of the concentration of the substances of which they are composed. Following this clue, one finds that the eggs of most animals possess a visible *axial*

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[1] *E. Hering.*

differentiation,[1] which is expressed in the fact that the density of some chemically identifiable inclusions varies in the direction of one outstanding diameter, which is then called the axis. The cytoplasmic substances specifically connected with developmental function can roughly be divided into two groups: *building* materials and *reserve* materials. The former are the active protoplasmic compounds (called *oöplasm*) whose transformation is going to give rise to the various structures of the developing organism, while the latter merely represent some sort of nutriment stored away in the egg to be later resorbed, digested, and assimilated by the proper protoplasmic substances.

Yolk.

Most of the heavier materials rich in proteins which we call *yolk* belong in the second class. The amount of yolk present in an egg is in some proportion to the length of time the developing individual requires before it can secure its own food. The quantity of yolk present in the eggs of forms possessing early and simple free-living larval stages (e.g., echinoderms) is practically negligible when compared to the large bulk present in eggs which hatch comparatively late in development (e.g., birds).

Wherever it is present in appreciable amounts (excepting insects), yolk tends to assume an *eccentric* position in the egg (so-called *telolecithal* eggs). In a number of cases this eccentricity can be ascribed to the fact that yolk is pumped into the egg at the side at which the latter is attached to the ovary, the yolk particles remaining crowded near the region in which they have entered. The pole of greatest yolk density is called the *vegetative* pole; its antipole, at which true oöplasmic substances reach the greatest concentration, is known as the *animal* pole. A line connecting both marks the *egg axis.* Since yolk is heavier than the rest of the egg content, the center of gravity of the whole egg lies nearer the vegetative than the animal pole. Therefore, eggs which can rotate freely (e.g., frog's eggs) orient themselves so that the axis is approximately vertical with the vegetative pole downward. When a frog's egg is inverted, it rights itself. If it is inverted but prevented from rotating back as a whole, then the heavier yolk begins to stream down on the inside while the lighter materials rise to what has now become the top of the egg (see example 35). This rearrangement of the egg content according to specific gravity proves that the interior is in a *semiliquid* condition.

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[1] Comparative treatment of the promorphology of the egg: *Korschelt* and *Heider*, 1936; *Wilson*, 1925.

Centrifugation

Under ordinary circumstances a relative condensation of yolk in the lower portion of the egg is all that gravity can achieve. The great inner friction of the viscous protoplasm prevents the comparatively slight differences of specific gravity involved from effecting a complete separation within the time available. Other egg inclusions differing still less in specific gravity do not undergo any perceptible segregation at all. Only by increasing the gravitational forces enormously through centrifugation (see p. 144), can one produce a nearly complete separation of the egg content into a number of constituent phases (Fig. 32). A sea urchin egg after such treatment is stratified into sharply delimited layers which, listed in the order of their specific gravities from light to heavy are the following: (1) oil, (2) homogeneous watery layer with high protein content, (3) mitochondria, (4) yolk, (5) pigment. Since only such a violent centrifugation can fractionate the substances of the egg content, it is clear that under *natural* conditions the effect of gravity can be disregarded as a potential source of finer chemical localization within the egg, although it can produce some crude localization by influencing the relative distribution of yolk and oöplasm.

Core-crust differentiation.

The question, as to whether there is any finer chemical localization in the cytoplasm at all, does not seem to admit

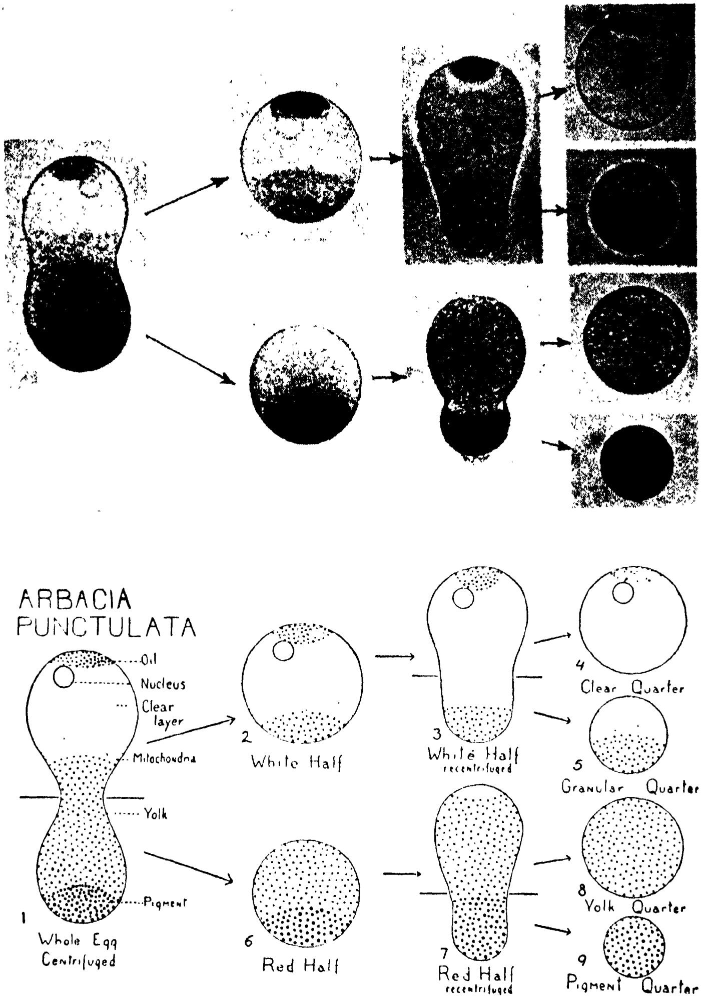

FIG. 32. Fragmentation of the sea urchin egg (*Arbacia punctulata*) by centrifuging. (*E. B. Harvey*, original) Top: photographs of the living object. Bottom: explanations.

First column: Egg stratified; light and heavy portions begin to separate.
Second column: Light and heavy halves have separated.
Third column: Light and heavy halves elongate.
Fourth column: Halves have broken apart into quarters.

of a uniform answer. Judging on the basis of the very fragmentary data at hand, one would be inclined to deny the existence of a rigid order in the *interior* of the egg but would concede it to the egg *surface*. This seems to be the view most easily reconcilable with what we know about the physical structure of the egg. As we have just stated, the interior of the egg is in a semiliquid condition. Toward the surface the physical state of the cytoplasm approaches more that of a solid, or since we are dealing with a colloid system, of a gel;[1] consequently, the physical conditions in the surface are more apt to keep apart substances of different chemical constitution than are the conditions in the interior where diffusion can level down existing differences.

The problem of egg structure.

The problem of chemical organization is indeed inseparably connected with the problem of physical structure. It would be easy to envisage distinct chemical laboratories in the cytoplasm laid out neatly according to a definite spatial plan, if only there were a solid framework to serve as scaffolding. As the matter stands, there seems to be no such framework in the egg. It is true that egg protoplasm, when studied in histological preparations, gives the impression of being pervaded by a spongy network of fibers; but this has long been recognized as an artifact produced by the coagulating effect of the fixing agent (see p. 124), and is no more representative of the living condition than a hen's egg hard-boiled resembles the fresh condition. Exploration of the living egg has failed to reveal the presence of a skeleton in its interior,[2] and overwhelming evidence speaks against the assumption of any *solid mechanical* structure in the cytoplasm (cf. p. 233). This does not preclude the possibility of a *dynamic* structure, that is, a definite pattern into which the diverse molecular groups would force one another by mutual specific repulsions, attractions, and other interac-

[1] *Just*, 1939.

[2] *Chambers*, 1924.

tions; even this assumption, however, would rest on wholly hypothetical grounds.

All this negative evidence pertains only to the *core* of the cytoplasm. With regard to the *crust*, we cannot be equally positive. Several examples will be reported later showing that important developmental events frequently result from the activity of regions originally situated in circumscribed parts of the surface of the undivided egg. The *gray crescent* of the amphibian egg (p. 351), the *yellow crescent* of the ascidian egg (p. 229), the *polar plasms* of annelids (p. 238), the *vegetative field* of echinoderms (p. 227) are cases in point. Many of these surface districts of later prominence are distinguished by peculiar pigmentation or different colloidal consistency or other features indicating that they are different from other parts of the surface. The main point which makes this surface topography suspect of being directly linked to later developmental organization is that the mosaic of recognizably different sectors occurs in a typical pattern, rather than as random mottling. As will be shown below, there is true developmental significance to the "geographical" configuration of the egg's crust. The difference between the properties of the two polar regions is particularly marked. It has been stated that there is higher metabolic activity at the animal pole,[1] that the pH there is higher (indicating alkaline state),[2] and that there are still other signs of physico-chemical differences between the upper and lower hemispheres.

Thus, the cytoplasm of the egg undeniably contains some primitive organization in its surface. But this organization is simple beyond comparison, when contrasted with the enormous diversity of local characters appearing in the later course of development, as listed in Part One.

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[1] *Bellamy* (1919) has deduced axial metabolic differences from susceptibility experiments. *Brachet* and *Shapiro* (1937), however, determining metabolic activity directly, were unable to find any difference between the animal and vegetative half of an *undivided* frog egg.

[2] *Spek*, 1934.

The unfertilized egg.

In this condition, loaded with reserves and potential energy, the mature, unfertilized egg is in a state of suspense, to be released by the stimulus of fertilization. The egg can persist in this state, during which the fundamental vital functions (e.g., respiration) are carried on, for a certain length of time, but not indefinitely. Unless it is activated within a given time limit, its capacity to develop is lost. Eggs activated toward the very end of this period, so-called "overripe" eggs, often develop poorly and not uncommonly give rise to malformations.[1] This indicates that the unfertilized egg, although externally it appears to be at rest, in reality undergoes changes which make it gradually sink from optimum developmental capacity to a condition from which development can no longer occur. If one should disclose any physico-chemical changes attending this gradual loss of developmental power in unfertilized eggs, important conclusions could be drawn concerning the physico-chemical prerequisites of development. Studies of this sort have, in fact, revealed one significant correlate of developmental capacity, namely, the *colloidal condition of the egg.*

Colloidal changes in the mature egg.

The *colloids* of the mature egg are in a state of comparatively high dispersity (see p. 29). The ultramicrons are small and finely dispersed. This condition is unstable. As described previously (p. 32), a colloid left to itself gradually decreases in dispersity; the elements combine, become larger, and their distribution becomes coarser. This is exactly what seems to happen in the mature egg.[2] The colloidal particles of the egg begin to aggregate; the disperse phase becomes coarser, more consolidated, a trend which, as we know, reduces the plasticity of the egg material. It will be recognized that this colloidal change in the egg prior to fertilization is a reproduction in miniature of the changes of the tissue col-

[1] *Witschi,* 1934.

[2] *Runnström,* 1928c.

loids in ageing (see p. 34). *The mature egg, in a sense, ages.* The act of fertilization, or of artificial activation in parthenogenesis, comes to the rescue of the egg by reversing this trend, and if we may trust certain clues, the *activation* of the egg can be viewed as an act of *rejuvenation.*[1] The facts are that after fertilization the colloids of the egg are again in a state of higher dispersion, back in a condition from which they had gradually drifted in the pre-fertilization period.

Example 13.[2]

Physico-chemical changes at fertilization.

Sea urchin eggs were observed in dark-field illumination. This method permits to deduce the state of dispersion of the colloids from the color of the light which they refract. During the stages preceding fertilization, a gradual change in the surface layer from white to orange was observed. This change indicates that the colloidal texture of the surface is becoming coarser. *At the time of fertilization,* however, *a sudden reversal in the color was noted;* it returned to a silverish white, proving that an increase in the dispersion of the colloids had taken place. That these changes are not irrelevant for development, is demonstrated by the fact that only those eggs develop which have gone through the color reversal.

Thus *fertilization* restores *higher dispersity* to the egg colloids, but obviously this is not its sole effect. Fertilization does more to the egg than merely prevent it from congealing; it actually starts things moving. There is hardly any physico-chemical character we know of in the egg that would not change at this time. Not only do the colloids suffer an increase in dispersion, but they are thoroughly altered in other respects. Their faculty to persevere in the highly dispersed state, i.e., their *stability,* is considerably greater after fertilization than it was before.[1] The *solubility* of the egg proteins has markedly declined,[2] presumably owing to oriented coagulation of which the appearance of optical birefringence in the fertilized egg is strongly indicative.[3] The *viscosity* of the egg content has increased.[4] Further notable changes at fertilization in the *sea urchin* egg are changes of *electrical conductivity*[5]; an increase of *permeability*;[6] a jump of *respiratory activity*, as indicated by oxygen consumption, by a few hundred [7] to several thousand percent.[8] The increase in metabolic activity may be directly related to the increase in dispersity, although it is difficult to say which one should be regarded as the primary effect of activation. All these phenomena have been observed to occur, no matter whether activation was brought about by fertilization or by artificial parthenogenesis.

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[1] Introduced in a figurative sense by *Bütschli* (1876) with special reference to protozoans, the idea of a rejuvenating action of fertilization has been accepted by *Hertwig* (1920) and assumes concrete meaning in the light of established colloid-physical facts.

[2] *Runnström,* 1928c.

The mechanism of activation.

While all this is going on, the only visible change is a certain shrinkage of the egg and the lifting of the so-called fertilization membrane from the surface. Since the interpretation of the multitude of physico-chemical data concerning fertilization has not kept pace with the rate of their accumulation, we are still far from able to piece them together into a coherent story. At any rate, the story consists of two parts: first, the provocation through a variety of agents of the described physical and chemical changes in the egg, and second, the connection between these changes and ensuing development. No common denominators have yet been found for either group. It is evident that the key unlocking the activation mechanism in the unfertilized egg must be something which all natural and artificial activating agents have in common. Withdrawal of water from the egg has been regarded as the crucial factor;[1] this does not agree, however, with all known facts. Whatever the inciting factor may be, its primary effect seems to be the liberation of some *acid* in the egg.[2] This occasions further physico-chemical changes as a result of which chemical reactions of unknown character and increasing complexity take place.

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[1] *Runnström*, 1928d, 1932.

[2] *Mirsky*, 1936.

[3] *Moore* and *Miller*, 1937.

[4] *Heilbrunn*, 1927. As there is generally an inverse relationship between viscosity and dispersity, the parallel increase of viscosity and dispersity seems incongruous. It has been suggested, however (*Runnström*, 1928d), that although the actual size of the ultramicrons decreases (increased dispersity!), their *effective* size, as far as inner mobility (i.e., reciprocal viscosity!) is concerned, becomes larger owing to an increase in their water binding capacity (*Spek*, 1924) which enlarges the shell of water around each element.

[5] *Gray*, 1916, 1922.

[6] *Lillie*, 1917, 1918; for a review of the physico-chemical changes at fertilization see also *Fauré-Frémiet*, 1925.

[7] *Warburg*, 1908; *Shearer*, 1922; comprehensive review in *Needham*, 1931.

[8] *Örström*, 1932.

The task of drawing a consistent picture of activation is rendered the more difficult as many of the observations made on one object are not representative of other forms. For instance, the sudden rise in permeability and respiration characterizing the sea urchin egg after fertilization is not observed in the closely related egg of the starfish,[3] and in some other forms (e.g., Chaetopterous) there is even a drop.[4] Whereas some insects, tunicates, fishes, and amphibians behave like the sea urchin, some worms and molluscs line up with the nonconforming starfish. In view of this situation, the *rise of metabolic rate cannot possibly be considered as an essential attribute of activation.*

Cellular and developmental effects of activation.

The interpretation of activation is further complicated by the fact that the fertilized egg engages in a number of processes of different order simultaneously. The fertilization membrane forms, the nucleus prepares for division, in the cytoplasm the mitotic apparatus forms, currents displace cytoplasmic areas, the cytoplasm cleaves, and differentiations are initiated. It is obvious that the physico-chemical changes

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[1] *J. Loeb*, 1913.

[2] *R. Lillie*, 1931; *Runnström*, 1932.

[3] *J. Loeb*, 1902.

[4] *Whitaker*, 1933.

observed at fertilization may be responsible for any one of these processes or for all of them. Some of the colloidal changes noted concern only the formation of the mitotic spindle and the preparation of the nuclear division, others pertain to the cleaving of the cytoplasm, still others to the segregation and dislocation of egg materials. Inasmuch as nuclear division and cytoplasmic fission are common to all cells and in no way peculiar to the developing egg, that part of the activation reaction which produces the segmentation of the egg is not necessarily pertinent to the strictly developmental phenomena. In other words, one must admit the possibility that a number of those conspicuous modifications and revolutions occurring in the egg after fertilization are merely factors of *segmentation* of no direct bearing on *differentiation* and *organization* in the strict sense. This possibility will be discussed later.

CLEAVAGE

Segmentation and organization.

In making a distinction between factors determining *segmentation* and factors determining *organization* of the embryo, we introduce an assumption for which we have as yet offered no evidence. Might it not be that the first step of development consists of nothing more than mere segmentation of the egg and that the conditions arising from this step are the determining agents for the subsequent stages of embryo formation? As a matter of fact, at one time there were numerous adherents to this viewpoint among embryologists. They maintained that there is an orderly mechanism of cleavage so arranged that different properties and potencies are suitably apportioned to the various blastomeres during successive cleavage divisions, and that these blastomeres then proceed, in line with their assignments, to give rise to corresponding fragments of the embryo, so that the final organization of the embryo would be a direct consequence of a *definitely regulated mode of cleavage.* Opinion was divided, as to whether the supposed regularity of cleavage was a matter of the *nucleus* or of the *cytoplasm.*

Weismann [1] was the protagonist of the former view. He thought that the heterogeneity of the embryo is brought about by an orderly and minutely regulated spatial distribution of successive generations of nuclei produced by qualitatively unequal divisions. The nucleus of the fertilized egg was supposed to contain all the necessary "determinants" for the differentiation of the embryo. In the first nuclear division, the determinants for the right and the left half of the organism would be segregated from each other. In the next division the anterior and posterior determinants of each half would separate. During the following division perhaps dorsal and ventral determinants would move apart, and so forth. By the regular spatial distribution of the nuclei through oriented mitoses and the attending orderly sorting out of "determinants," each nucleus was thought to arrive at a predestined place, equipped with a "purified" single potency. It is obvious that such a mechanism of development would require nuclear segmentation and distribution of clock-like precision; for the slightest accidental disarrangement of the prearranged mode of division or pattern of segregation would cause corresponding confusion in the developmental process. Although *Weismann* made allowance for this possibility by postulating the presence of regulating reserve mechanisms in each nucleus to be activated in emergency cases, his theory is contradicted by many experimental facts.

Example 14.

Modification of the nuclear cleavage pattern.

In the cleaving frog's egg the first two fissures occur along meridional planes in the manner in which one divides an apple into fourths. The third division, leading from the four-cell to the eight-cell stage, occurs parallel to the equator, perpendicu-

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[1] *Weismann,* 1892.

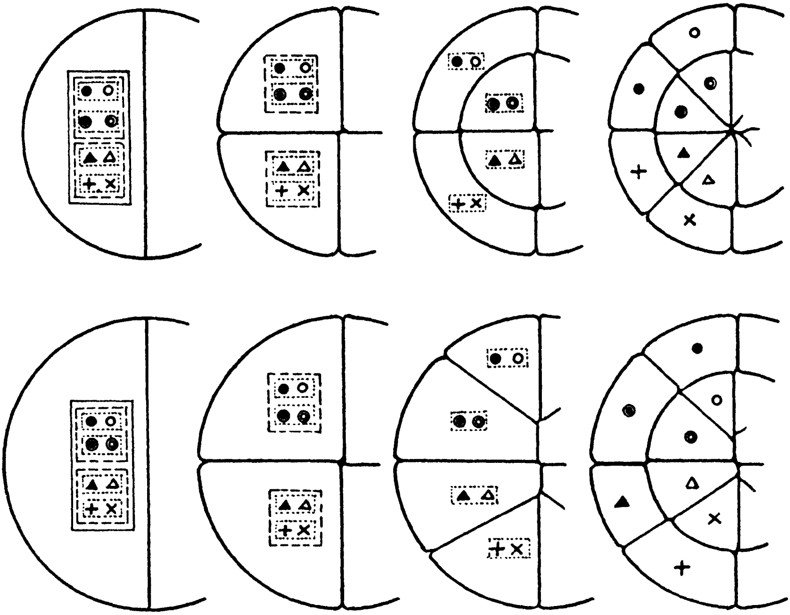

FIG. 33. Distribution of segmentation nuclei in frog's eggs cleaving normally (upper row) and under vertical pressure (lower row). Polar views.

The diagrams are based on the assumption that the nucleus of the fertilized egg contains a definite array of potencies which would be segregated by a definite sequence of unequal nuclear divisions. The potency of each nucleus of the 16-cell stage is designated by a separate symbol and the supposedly regular sequence of their gradual release is indicated by the successive removal of common inclosures. Thus, for instance, the second cleavage step (from 2 to 4 cells) separates the sets with broken outlines; the third step, the sets with dotted outlines; the fourth step, the individual symbols. This sequence of steps is the same in the upper and lower rows. It can be seen that the nuclear assignments of the blastomeres of the 16-cell stage would be entirely different in the unpressed and the pressed eggs.

lar to the first two planes; it separates four upper (animal) from four lower (vegetative) blastomeres. One knows that the four animal blastomeres will contribute mainly to the formation of anterior and dorsal parts of the embryo, while the four vegetative blastomeres will give rise to ventral and posterior regions. Therefore, if the division of the nuclei proceeded according to a fixed and regular scheme, the third segmentation division would separate nuclei with antero-dorsal assignments from nuclei with ventro-posterior charges.

The pattern of nuclear distribution, however, can be altered experimentally by pressing the egg between parallel plates applied to the animal and vegetative poles. The egg axis is shortened and the egg flattened to disc-shape.[1] Under these conditions the third segmentation division follows again a meridional plane, just as the first two divisions did, instead of changing to an equatorial orientation. The resulting eight-cell stage consists of a single layer of blastomeres, all in one plane. Thus the supposed antero-dorsal nuclei, instead of lying above the postero-ventral nuclei, come to lie side by side with them (Fig. 33). If the pressure is then released, each one of the eight cells divides into an upper and a lower half so that now both the upper and the lower halves of the germ share evenly in the derivatives of both the presumably antero-dorsal and postero-ventral nuclei. This not only *brings the nuclei into abnormal spatial distribution* but also *pairs them with strange sectors of cytoplasm* with which normally they would not have been connected. Yet in spite of the thorough and irreparable disarrangement and confusion of the nuclear distribution thus produced, *development of the germs proceeds normally* and shows no evidence whatever of the upset of cleavage order.[2]

It is obvious that such a result could never have been obtained if individual nuclei arising from segmentation divisions drew differential assignments for the control of limited portions in the supposed developmental mosaic. One must conclude, therefore, that *all segmentation nuclei are to all purposes identical in character and equivalent in potency.* This conclusion is corroborated by the following experiment.

Example 15.[3]

Further proof of the equivalence of segmentation nuclei.

A fine ligature is slung around the fertilized egg of a newt so as to constrict it into two halves, of which one contains the

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[1] *O. Hertwig*, 1893.

[2] About similar experiments in other forms, see *Morgan*, 1927.

[3] *Spemann*, 1914, 1928.

egg and sperm nucleus, while the other contains no nucleus at all. The constriction is effected so that there is just a small protoplasmic bridge left connecting the two parts (Fig. 34). Presently the part containing the fused nuclei begins to segment in the normal way. The other, non-nucleated, part at first fails to segment but remains attached to the blastomere nearest to the constriction. Occasionally during one of the later divisions it happens that a daughter nucleus of this blastomere slips through the narrow protoplasmic peduncle, escapes into the hitherto unsegmented part of the egg and incites it to divide. Both halves may develop, and the result is the formation of *two whole embryos* — one from the originally nucleated part of the egg, and the other from the delayed portion, despite the

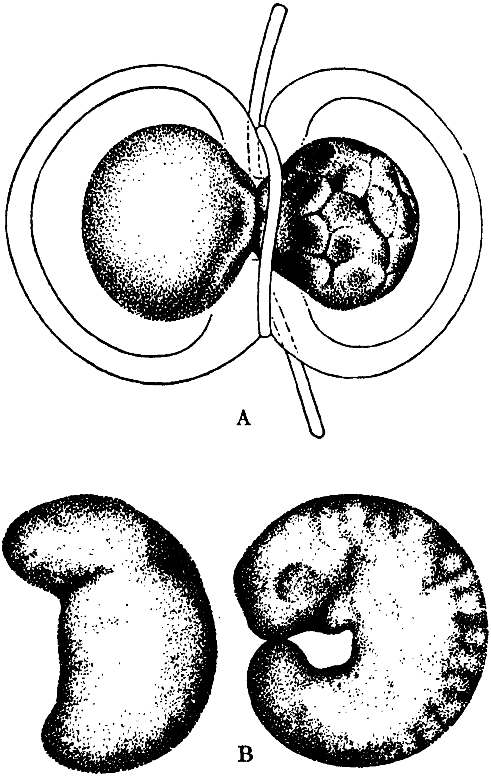

Fig. 34. Twinning of newt embryos combined with delayed nucleation of one half. (From *Spemann*)

A, Constriction of the egg along the first furrow during the 2-cell stage has left the copulation nucleus in the right half. This half has cleaved. At the illustrated stage the nucleus of the blastomere nearest to the “[illegible]leneck” escapes into the unsegmented left half which presently begins to cleave.

B, Twin embryos developed from A. The lag of the left partner owing to the initial delay still is appreciable.

fact that this latter had only a fractional derivative of the original egg nucleus at its disposal.

The capacity of bisected eggs to give rise to twins will be commented on later (p. 302). The present experiment should merely serve to illustrate that the development of a complete animal can be obtained from an egg fragment which has never received more than a fraction of the original egg nucleus.[1] The nucleus of a blastomere of the sixteen-cell stage was found to be entirely adequate to permit complete development.[2] Nucleation of the delayed portion at still later stages fails to yield development, but this failure must be ascribed to the gradual loss of developmental ability in the cytoplasm rather than to deficiencies of nuclei of later nuclear generations.

These experiments prove that the pattern according to which the segmentation nuclei are divided and distributed can have no bearing on the localization of embryonic parts and on the appearance of progressive inhomogeneity in the germ. *The successive generations of nuclei*, as they arise during segmentation, *are interchangeable and, therefore, equivalent.* Consequently, the concept of unequal nuclear division as mechanism of differentiation must be rejected. It has, moreover, in the meantime been discredited by the findings of geneticists and cytologists.

Is cytoplasmic cleavage an instrument of differentiation?

Having ruled out the nuclear division pattern as an instrument of embryonic organization, we must next turn to the *pattern of cytoplasmic fission* as a possible factor. Considering that the cytoplasm possesses a certain spatial organization, one understands that cytoplasmic cleavage might very well serve to fence off areas of different developmental properties which might subsequently turn into embryonic organs. There is no doubt that different blastomeres often receive allotments of cytoplasm of different character. The only question is whether this fact is of fundamental significance for the formation of the embryo. The following discussion will deal more fully with this question.

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[1] See also *Fankhauser*, 1930.

[2] An analogous result was obtained in insects (*Seidel*, 1932). One of the first two segmentation nuclei was killed by ultraviolet radiation (see p. 159). The descendants of the remaining nucleus spread evenly over the whole blastoderm, and a normal embryo developed. Sometimes, however, the local injury produced by the irradiation prevented the shifting of nuclei into the "enucleated" half until at some later cleavage stage. But even a nucleus of the eighth cleavage generation (stage of 128 nuclei), when it finally managed to slip past the obstruction, could still successfully resettle the delayed half with its descendants, and the whole egg produced a normal embryo.

The problem of cytoplasmic continuity of the germ.

First, is the cleavage of the cytoplasm really as complete as is the division of the nuclei? Does the erection of cleavage partitions sever all protoplasmic continuity among blastomeres? In some forms it does, in others it does not. In neither case does it seem to matter to development. In certain forms the blastomeres are said to be connected by protoplasmic bridges, so-called *plasmodesms.* In some eggs (e.g., birds, teleosts) the early segmentation stages (Fig. 35) lead only to very imperfect separation of the blastomeres, and complete individualization of cells does not take place until in later stages. In the insect egg the fertilized nucleus, which lies in the center of the egg embedded in a small area of oöplasm, produces a flock of segmentation nuclei by a series of successive divisions. These nuclei then move to the surface of the egg which up to that time has not shown any trace of cytoplasmic fission. A continuous layer of oöplasm (blastoderm) covers the surface of the egg, and in this layer the nuclei enter. Only after this has happened, partitions begin to form which outline the territories of protoplasm belonging to each nucleus against one another. Here the egg is in a true syncytial (symplasmic) condition up to the very end of segmentation. In contrast to this type, one finds eggs, particularly among the forms containing only small amounts of yolk, in which the arrangement of the blastomeres is so loose that there is no suggestion of a more intimate connection among them than mere contact. Occasionally, the separation is complete in the interior of the germ, but intercellular continuity is maintained by a common layer of surface protoplasm enveloping the whole group of blastomeres.[1]

Making due allowance for the impossibility of deciding an issue of this sort by microscopic evidence, for reasons which have already been explained (p. 125), one can still build a strong case against the assumption of universal presence of plasmodesms between blastomeres. Furthermore, the fact that blastomeres can be experimentally separated from each other and then be recombined without impairing the normal character of the subsequent development (p. 270), proves that such *cell bridges*, even if they existed under normal conditions, *cannot be essential and indispensable for development.*

The whole matter loses some of its obscurity as soon as one visualizes the living protoplasm not in the light of classical morphology but under the viewpoint of general physiology, where the picture of the living "cell" necessarily includes that outermost layer which we call "cell membrane." [2] In this broader view, the setting up of cell boundaries during cleavage with the attending establishment of new interfaces appears as an instrument for the diversification of cell relationships rather than as a disruptive measure. Cell membranes, while still permitting specific exchange and interaction, do at the same time insure a certain degree of independence among the individual cells.

What determines cleavage patterns?

The *pattern of segmentation* is determined by three factors: (1) the *rate* at which segmentation proceeds; (2) the *orientation* of the cleavage planes (i.e., the direction in which the dividing cells separate); and (3) *shifts* of cells relative to each other. The orientation of a cleavage plane, in turn, is a function of the position of the mitotic spindle. Hence, all factors influencing the orientation of the spindle affect the cleavage pattern.[3] On the whole, the orientation of the spindle depends upon the constitution of the protoplasmic area in which it forms, and this connection has been expressed in a number of rules, as follows.[1]

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[1] *Gray*, 1924.

[2] Cf., for instance, *Heilbrunn*, 1937.

[3] Further information in *Wilson*, 1925; *Fauré-Frémiet*, 1925.

The spindle assumes a position in the central portion of the true oöplasmic material. In eggs in which, owing to the presence of heavier yolk, the oöplasm is concentrated in the upper portion (p. 189), the spindle forms eccentrically, nearer the animal pole. *The long axis of the mitotic spindle,* i.e., the line connecting the two astrospheres, *coincides with the longest diameter of the oöplasmic area,* and *the plane of fission runs perpendicular to the axis of the spindle.* In telolecithal eggs the oöplasm, which forms a cap or disc in the animal hemisphere, has its shortest extension in the direction of the egg axis. Consequently, the first spindle assumes a position at right angles to the egg axis, and the first cleavage plane, being perpendicular to the spindle, must again include the egg axis, i.e., follow a meridional plane. For similar reasons, the second cleavage plane must also be meridional and cross the first one at right angles (Figs. 35, 40, 43). In eggs with very small to moderate amounts of yolk (e.g., echinoderm or amphibian eggs) the oöplasmic area of each blastomere of the four-cell stage is elongate parallel to the egg axis; therefore, the spindles preparing the third cleavage division orient themselves now in this direction and produce the first fission across the egg axis at, or parallel to, the equator (Figs. 36, 43). In extreme cases, however, where the egg contains excessive amounts of yolk and the oöplasm is a thin disc on top of the yolk (e.g., in birds, teleosts), the spindles continue to assume positions parallel to the surface for many more divisions (Fig. 35).

The agents operating in the formation and orientation of the spindle have received some attention. Obviously the spindle is a product of oriented cytoplasmic coagulation; once formed, it is a rather coherent body, for by micro-

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[1] Known, after their author, as *Hertwig*'s rules (*Hertwig*, 1920).

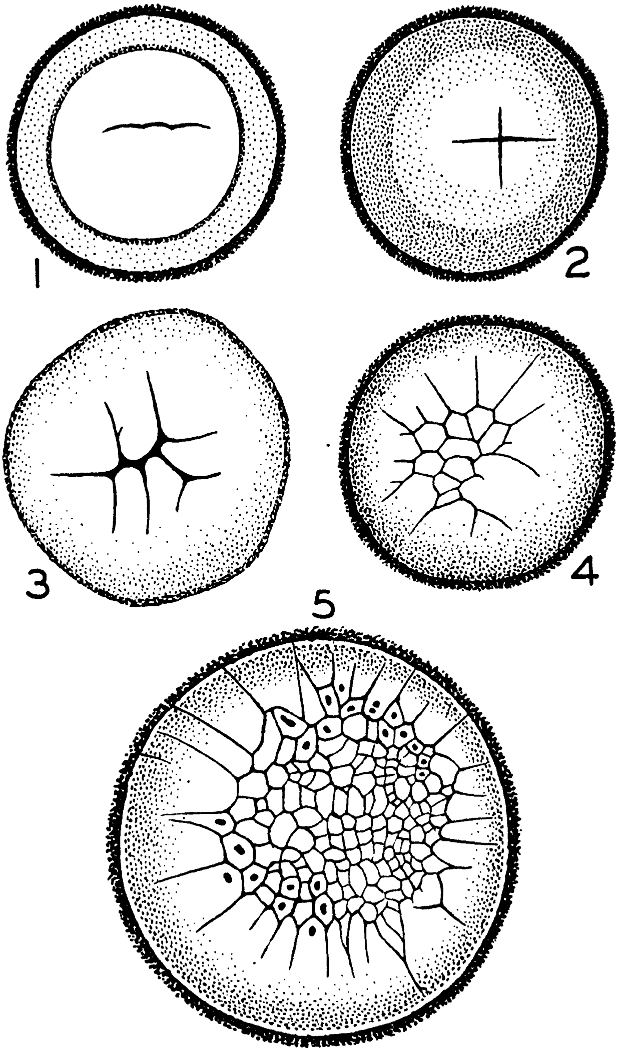

FIG. 35. Cleavage stages of the chick blastoderm. (Redrawn from *Lillie* after *Kölliker* and *Patterson*)

manipulation it can be moved around as a whole inside the egg.[1] Protoplasmic currents affect its orientation, and a definite symmetry relation between spindle and cell body is enforced by physical factors not yet fully explored. Generally speaking, *the mechanical conditions prevailing in the cytoplasm appear to exert some control over the orientation of the mitotic apparatus* and, hence, over the direction in which the cytoplasm is constricted and divided.

What determines the first cleavage plane?

These rules prescribe that in an egg in which the oöplasm is uniformly distributed around the egg axis the first spindle must lie radially with regard to the axis; but they do not specify the particular radius with which the spindle is to coincide. Thus the question arises as to what factor determines among the infinite number of radial positions possible the one that is actually assumed. In certain forms the sperm was held to be the determining factor. In the frog's egg the first furrow was originally thought to cut through the point at which the sperm has entered the egg. A more thorough investigation [2] suggested that it was not so much the entrance point of the sperm as the course which it takes within the egg that has an orienting influence on the mitotic mechanism. After penetrating through the superficial layers of the egg, the sperm moves toward the egg nucleus leaving behind a track which is known as the "*copulation path.*" This line was supposed to fix the position of the first cleavage plane. As a matter of fact, this relation seems to hold true in a majority of cases. Perhaps even more significant, however, is the fact that this rule is by no means rigid.[3] In a sufficiently large material, many cases come to one's notice in which the *first cleavage plane and the copulation path of the sperm do not even approximately coincide.* Therefore, the coincidence can be regarded only as *preferential,* not as absolute.

[1] *Chambers,* 1924.

[2] *Roux,* 1887.

[3] *Weigmann,* 1927.

This, of course, rules out the original contention that the position of the first cleavage plane is primarily a matter of the sperm entrance. The situation, somewhat simplified, seems to be as follows: Any local inhomogeneity in the colloid-physical state of the cytoplasm (liquefaction, current, etc.) may create a line of lowered resistance along which the fission of the egg could occur. Such local changes, while usually following in the wake of the penetrating sperm (which explains the preferential coincidence between copulation path and cleavage plane) can just as well develop from accidental local disturbances not connected with the sperm, and in this case the cleavage plane will appear in a random radius. In other types of eggs, e.g., in the echinoderms,[1] there is no indication of an influence of the sperm on the orientation of the first cleavage plane; furthermore, there can be no question of any such influence in the case of parthenogenetically developing eggs. Therefore, we conclude that, while the constitution of the egg causes the first cleavage plane to lie meridionally, the choice of a definite meridian is more or less accidental, with the sperm frequently supplying the accident.

Considerable importance was attached to this question at the time when there was still a widespread belief that development consists of a rigid sequence of simple steps, each step related only to the immediately preceding and the next following one. Of these steps, the entrance of the sperm was thought to be the first; hence, the emphasis placed upon it. At the rate at which increasing knowledge made that oversimplified concept of development appear untenable, interest in the problem of just how the cleavage planes were fixed declined. One has come to realize that it does not matter very much to the development of the individual whether the cleavage planes are oriented one way or another.

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[1] *Hörstadius*, 1928.

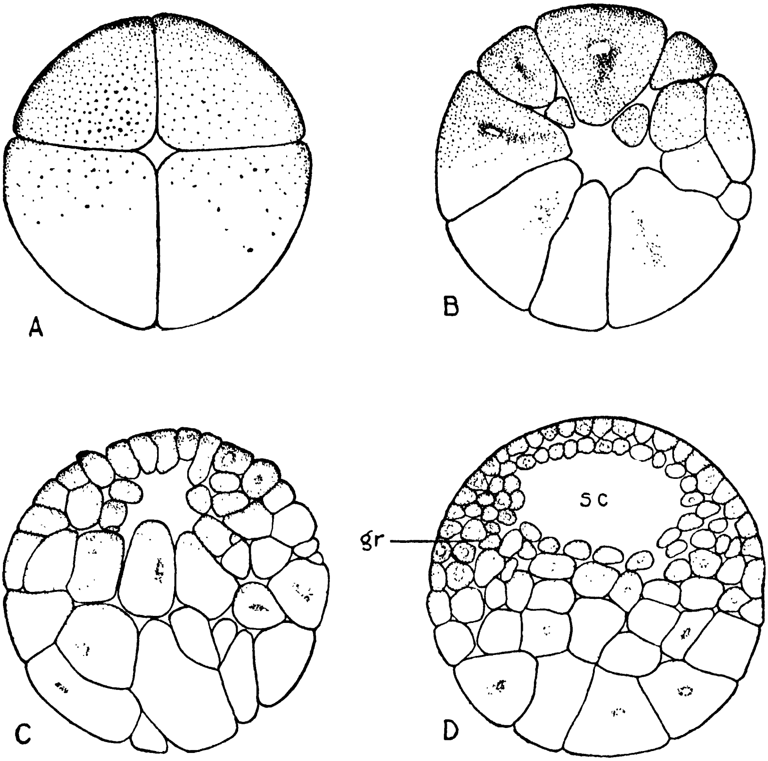

FIG. 36. Median meridional sections of four cleavage stages of the frog. (From *McEwen*) Note irregularity of blastomeres and size gradient of cells in the direction of the egg axis.

gr, Dorsal part of marginal zone derived from center portion of gray crescent.

sc, Blastocœle.

Determinate and indeterminate cleavage.

The second cleavage plane is invariably perpendicular to the first; the third cleavage plane usually bears a fixed relation to the former two, and in some forms the geometrical order remains very definite and regular even during the following divisions. However, on the whole, the variability of the details of the cleavage pattern becomes increasingly greater during the later stages, and after some time the initial regularities are no longer discernible (Figs. 35, 36).

Different groups of animals differ markedly in regard to the time when this happens. Those whose cleavage follows a fairly definite pattern into late stages are called *determinate* cleavage types; those in which the cleavage pattern is, even during early stages, subjected to a considerable amount of variation, are called *indeterminate* cleavage types. Neither extreme is ever realized in pure form, but animals can be classified according to whether they come closer to one or the other type.

Protoplasmic influences on spindle orientation.

While frequently the rules outlined above account adequately for the orientation of cleavage planes, the mode of cleavage of certain invertebrate eggs does not conform with the scheme. In the groups exhibiting the so-called *spiral* type of cleavage, including annelids and molluscs, the cleavage spindles lie neither parallel to nor at right angles to the egg axis; they slope at an angle, all in the same sense. The direction of the slant alternates from division to division, so that if in one division all spindles are inclined to the right, during the next division they will be inclined to the left. This deviation of the spindles from the position which they should assume in accordance with the shape of their oöplasmic area indicates that there are certain *intrinsic asymmetrical structures or forces inherent to the protoplasm* which guide the pattern of cleavage in these forms. Even in this case, however, the peculiar pattern of cleavage is clearly a *result* of an underlying organization of the egg protoplasm rather than a *factor* through which such an organization might be produced. Asymmetrical cleavage results from asymmetrical conditions in the protoplasm, rather than vice versa.

Cohesion and shifts of blastomeres.

Blastomeres emerging from a division rarely stay in the place where they arise, nor do they retain the nearly spherical shape in which they come out from the fission. During the last phase of mitosis the daughter cells round off and thus tend to pull apart, but presently they approach each other again as if drawn by active force. There are several factors acting synergically to hold the blastomeres together. One factor is the membrane enveloping the egg, but even if the membrane is experimentally removed, the germ does not fall apart. The blastomeres cohere owing to a certain stickiness of their surfaces. In marine eggs this stickiness seems to be conditional upon the presence of calcium ions in the sea water, because in the absence of this element the coherence is lost and the blastomeres drift apart.[1] A very thin layer of more consolidated protoplasm which has been reported to cover the surface of some eggs as a sort of elastic skin is an adjuvant factor.[2] Finally, *surface tension* promotes cohesion of identical bodies within a strange medium.

With these forces in operation, the individual blastomeres cannot always retain the positions into which they have come to lie after division. They shift upon one another until their common surface most closely fits the form of a sphere, or in the case of partial cleavage, part of a sphere. In this regard semiliquid blastomeres behave precisely like anorganic droplets, or bubbles of gases surrounded by a liquid film. The same laws which control the arrangement of soap bubbles under the action of surface tension are expressed in the arrangement of blastomeres in eggs of the total cleavage type. According to these laws, formulated by *Plateau*, not more than three planes can meet at any one edge, and not more than four edges at any one point. The angles under which the planes and edges meet and the curvature of the inner surfaces are also defined by these laws. The individual cells of an aggregate of blastomeres slide into the stable positions prescribed by the laws of surface tension, just as do the bubbles of a foam. A large number of cleavage configurations can be explained on this basis. There remain, however, some observations on oriented blastomere movements which are refractory to this interpretation and indicate that cell shifts can be caused by more specific forces than mere surface tension. Nothing is known about what these forces are and how they operate.

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[1] *Herbst*, 1900.

[2] *Gray*, 1924.

Cleavage rate.

The *rate* at which cleavage proceeds in a blastomere is a function partly of the character of its protoplasm, partly of its non-protoplasmic content, such as yolk, and partly of controlling influences exerted by the other blastomeres. As a rule, the intervals between divisions are the longer the more yolk a cell contains in proportion to its plasma.[1] This is because yolk takes no active part in the mechanism of mitosis; as mere ballast, it retards the division. If the amount of yolk is excessive, then cleavage, which begins in the oöplasmic (animal) area, is not only more and more retarded, the further it proceeds toward the vegetative pole, but often entirely fails to cut through the inert yolk mass, thus creating a syncytial condition. Since the concentration of yolk increases from the animal toward the vegetative pole, cells at the animal pole generally divide at a faster rate than those at the vegetative pole.

The size of blastomeres.

This naturally is of some consequence for the relative size of the cells. Since each segmentation division reduces the size of the cells, and as cell growth is absent during cleavage (p. 78), the size of individual blastomeres is roughly in inverse relationship to the number of past divisions. Therefore, cells in the more rapidly dividing animal portions where the oöplasm is not encumbered with yolk are smaller than the more vegetative cells which are laden with yolk and cleave more slowly.

While unequal size of blastomeres is frequently a direct result of inequalities in the division rates (e.g., in the frog, Fig. 36), it is by no means exceptional to find size differences among blastomeres which cannot be accounted for in such

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[1] This has become known as *Balfour's* rule.

a simple way. Sometimes intrinsic differences in the constitution of the cytoplasm force the cleavage apparatus into an eccentric and asymmetrical position in the cell. The egg of the ctenophore, in passing from the eight- to the sixteen-cell stage buds off eight cells in its animal region only a fraction as large as their sister cells (Fig. 40). Similarly, at the eight-cell stage of the sea urchin egg segmentation of the four vegetative cells leads to four small cells and four large cells, while the four animal cells divide equally (Fig. 43). In the eggs of the spiral cleavage type the four blastomeres into which an egg becomes subdivided during its first two cleavage steps bud off four small cells at their animal surfaces. These small cells then continue to divide more or less equally, while the original four large blastomeres diminish in size only gradually as they keep on budding off successive generations of small cells. In cases where there appear conspicuous and typical (i.e., not accidental) size differences among blastomeres, we call the largest cells *macromeres*, the smallest cells *micromeres*, and cells of intermediate size *mesomeres*. It must be kept in mind, however, that these are purely descriptive terms referring only to size relationship without implying homologies or similarities in other respects.

Blastomeres under common control.

In many forms a strict *synchronism* of cleavage is noted either among all blastomeres or within certain groups of them. In eggs of this kind the blastomeres divide simultaneously and pass through identical stages of mitotic activity at exactly the same time. This synchronism is usually confined to early segmentation stages. It indicates that the division of all blastomeres is controlled by some common signal, recurring periodically, much as a central electric plant synchronizes any number of clocks. Periodical changes in the permeability of the egg membrane, resulting in excitation through electrical depolarization of the surface could be advanced as a possible factor; like nerve excitation it would spread over the whole surface of the germ regardless of cell boundaries and could, therefore, well serve as a coordinator of blastomeral activities. Purely chemical agents seem to diffuse too slowly to deserve consideration as synchronizers. It has been suggested that some ultraviolet radiation of short wave length, generated by certain chemical reactions and purportedly a factor in mitosis,[1] might be the agent through which segmentation steps are synchronized. At present, little more can be said than that *blastomeres seem to be subject to some degree of common control.* In support one can cite the fact that in sea urchin eggs which have undergone treatment with alcohol or ether the nuclear divisions continue but are no longer synchronized, as if the treatment had paralyzed the controlling agent.[2] But no definite information as to the nature of the control has come to our knowledge.

The end of cleavage.

Conspicuous size differences that may have appeared in early cleavage stages (e.g., that between macromeres and micromeres) are usually levelled out during later stages (Fig. 43), with the exception of the general difference caused by the lagging of the vegetative behind the animal cells. Size differences due to different yolk content are more permanent than those due to temporary conditions in the plasmatic constitution (cf. Fig. 36).

Cleavage brings about a condition of the germ known as the *blastula.* In most forms the configuration is that of a hollow spherical body whose animal portion is completely divided into cells while the vegetative portion contains the yolk and is often incompletely cellulated. The cavity on the inside — the so-called *blastocœle* — lies the more eccentrically the more the mass of yolk exceeds that of oöplasm; it is filled with a colloidal material presumably exuded from the blastomeres.

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[1] *Gurwitsch,* 1932.

[2] *Polowzow,* 1923.

The role of cleavage in the differentiation of the embryo.

Having outlined in the foregoing some of the factors determing cleavage pattern, our next task is to appraise their significance for the formation of the embryo. Does the particular course of cleavage have a bearing on the organization of the embryo, and if so, how closely does the latter depend on the former? The hope to find a universal answer to this question cannot be long entertained when one thinks of the enormous latitude existing in the degree of definiteness of cleavage conditions and cleavage patterns among different forms of animals.

The possibility that an orderly sequence of *nuclear* divisions might be instrumental in establishing some embryonic organization had to be rejected (p. 201). This still leaves the problem as to whether the separation of *cytoplasmic* areas through cleavage might not be a mechanism to segregate and dispose properly parts of different developmental role. In order to settle this problem, one must ascertain, first, whether there is a *fixed part-for-part correspondence between blastomeres and definite embryonic portions,* and second, whether such a definite connection, if it should be found to exist, is an expression of *causal relationships* or of *mere coincidence.*

With regard to the first point the answer varies for different groups of animals, depending on whether they line up with the *determinate* or *indeterminate* cleavage types (see p. 210). In the former, circumscribed parts of the embryo can be definitely traced back to specific blastomeres; in the latter the relation is much more vague, and the exact portion of body produced by the descendants of a given blastomere varies from one specimen to another. As has been said before, the distinction between determinate and indeterminate types does not mean that all animals can be separated cleanly into two opposing camps; the terms refer merely to the two extreme ends of a series of gradations characteristic of different groups of animals. To the extremely determinate end belong the eggs of *annelids* and *molluscs* (with the exception of the *cephalopods*), and to the indeterminate end belong all highly telolecithal eggs, such as those of the *birds* and *cephalopods*, and also the *echinoderms* and *amphibians.*

Specific functions of blastomeres in determinate cleavage.

In the egg of an *annelid*, for instance, one definite blastomere of the twenty-four cell stage stands out from other cells of its generation by its singular character, behavior and fate. It arises through a division which is the very first one to get out of step with the other cells, being either precocious or delayed (depending on the species); it differs in size from the rest, and it assumes a peculiar position. To this single blastomere, known as the *mesentoblast,* the musculature of the segmented trunk of the later worm can be traced back. The mesentoblast moves into the interior of the germ, divides, gives rise to the so-called teloblasts; these by proliferation (p. 49) form the so-called mesoderm streaks (Fig. 88) which are the primordia of the trunk segments. But this is not all. Tracing the ancestry of that odd blastomere, one finds that the blastomere from which it is to be budded off, shows certain distinguishing features from the very beginning of cleavage. It is earmarked, in a sense, as the future mother cell of the mesentoblast, as if the material from which the mesentoblast and its derivatives are to be formed had been put aside already in the unsegmented egg. One cannot escape the impression that in this case *a definite embryonic part is represented in the undivided egg by a localized cytoplasmic portion*, and that segmentation serves the purpose of concentrating the materials destined for that part in a single cell. Inasmuch as assignments of similar definiteness may be ascribed to the other blastomeres as well, segmentation here appears to be engaged in separating cells with different developmental tasks from one another.

Indefinite function of blastomeres in indeterminate cleavage.

Eggs of the *indeterminate* cleavage type, on the other hand, (e.g., those of the frog or chick) show no such stereotypism of the prospective fate of a given blastomere. In these forms the general destination of certain large areas of the germ is roughly circumscribed, but the precise fate of an individual cell varies too much to be predictable. The blastomeres of indeterminate eggs, irregular from the beginning, become even more so as segmentation proceeds; their disposition becomes more and more random, and each case differs in detail from every other case. There is not much more regularity to the segmentation pattern of the embryonic disc of a chick egg than there is in the pattern of crackled enamel on a pot (Fig. 35). Consequently, individual eggs of this sort differ considerably as to the locations in the embryo where the derivatives of a given blastomere will lie, and it is difficult to see how a regular embryo could ever arise if every individual blastomere started with a definite embryogenetic assignment.

Are cleavage planes related to the symmetry of the future embryo?

But while the assumption of a *minute* correspondence between individual blastomeres and definite embryonic organs in indeterminate types must thus be rejected, there remains still the possibility that at least the *gross* characters of the embryo might be referable to corresponding general features of the cleavage pattern. It was assumed, for instance, that one of the *first two cleavage planes* lays down the future *plane of symmetry* (median plane, p. 52) of the embryo. The following observations have been advanced in support of this contention.

Example 16.[1]

In the two-cell stage of a newt's egg a ligature was slung around the egg along the first furrow as an identification mark of the position of the latter. The egg continued to develop, and later when the embryo had formed it was noticed that its median plane was either coincident with, or at right angles to, the plane marked by the ligature. In the latter case, the median plane corresponded to the second cleavage plane, which, as one knows (p. 206), is perpendicular to the first cleavage plane.

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[1] *Spemann*, 1901–1903.

This experiment seemed to indicate that the symmetry and bilateral organization of the future embryo are laid down as early as at the two- or four-cell stage, and that the first or, at the latest, second cleavage step either follows an already existing boundary between a left and right half of the egg, or, indeed, produces by its very occurrence the bilateral symmetry of the subsequent embryogenetic processes. The latter view, according to which the early cleavage pattern should actually establish the ground plan for the embryonic pattern, seems, however, irreconcilable with the following observations.

Example 17.[1]

If in the place of a ligature a vital stain is used to mark the position of the first cleavage plane, the results are much less uniform. Instead of an absolute correlation between the first or second cleavage plane and the median plane of the embryo, only a *preferential* coincidence between them is observed. That is, while in a majority of cases the two planes approximately coincide, there is a fair percentage of cases in which they are not so related (Fig. 37). Similar results have been obtained with the use of vital staining methods in another indeterminate egg, that of the echinoderms[2] (Fig. 37).

Lack of correspondence between cleavage pattern and embryonic symmetry.

The results of experiments 16 and 17 are ostensibly at variance. The discrepancy can be explained, however, if one concedes that tying a ligature around an egg might affect the developing embryo. The application of a vital stain, on the other hand, does not interfere with normal development (p. 129); only results obtained by this method can, therefore, be regarded as truly representative of the

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[1] *Smith*, 1922; *Vogt*, 1926; *Banki*, 1927.

[2] *v. Ubisch*, 1925; *Runnström*, 1926; *Hörstadius*, 1928.

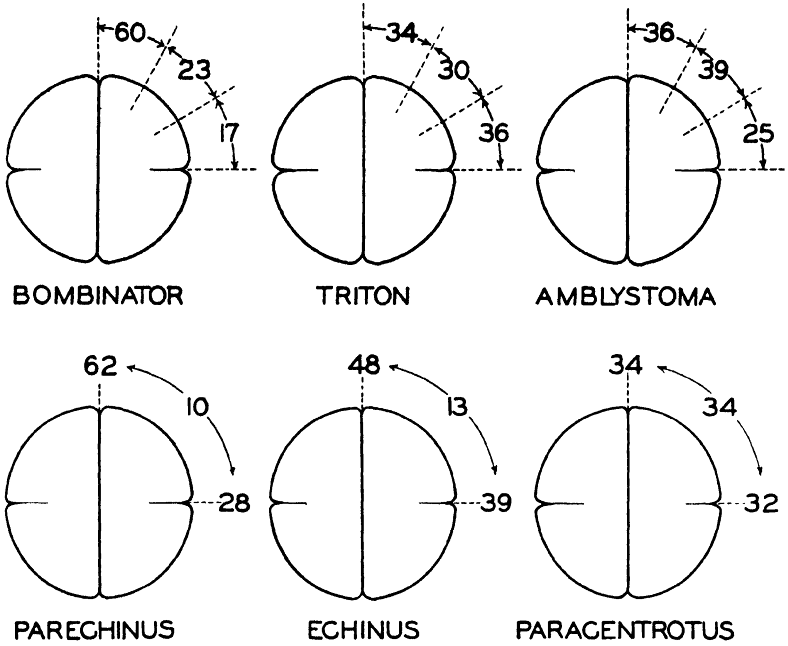

FIG. 37. Relation between first cleavage plane and median plane of embryo in amphibians (upper row; after *Vogt*) and echinoderms (lower row; after *v. Ubisch* and *Hörstadius and Wolsky*). The median plane may include any angle with the first cleavage plane. In the diagrams the frequency of the different angular positions observed (from 0° to 90°) is indicated in percentage figures (cf. also Fig. 51).

normal situation. Hence, we may accept the evidence of example 17, according to which *there is no absolute correlation between the symmetry of cleavage and the symmetry of the later embryo* in the indeterminate forms. This proves that cleavage cannot possibly be the specific factor through which the bilateral symmetry of the embryo is laid down. This relation evidently resembles that existing between the path of the sperm and the first cleavage plane (p. 208). In both cases the correspondence is preferential rather than absolute. Neither does the sperm entrance necessarily determine the first cleavage plane nor does the first cleavage plane necessarily define the orientation of the embryo.

Egg organization determines cleavage pattern rather than vice versa.

On the other hand, one cannot ignore the fact that there is sometimes a tendency of the embryonic median plane to be oriented along the first or second cleavage plane. The explanation seems to be that both segmentation of the egg and organization of the embryo depend upon a common third, but one more closely than the other. This common third is probably the basic organization of the egg prior to its segmentation. Let us suppose (1), that the later bilateral symmetry of the embryo is anticipated by a bilateral symmetrical disposition of substances and forces in the cytoplasm; for instance, by symmetrical protoplasmic currents, for which there is some evidence. Suppose further (2), that these same conditions, by means of the mechanical and electrical symmetry which they impose upon the egg content, force the mitotic spindle likewise to assume a symmetrical position in the egg. Then, the *collateral relationship between embryonic symmetry and cleavage symmetry* is plausible. The same differentiations and movements of the cytoplasm which establish the elementary framework for the future embryo concurrently impose definite spatial limitations upon the mitotic mechanism. The less definite the bilateral-symmetrical organization of the egg cytoplasm is when the egg prepares for division, the more at random will the spindle lie. Therefore, the variability revealed by example 17 must be ascribed to differences among individual eggs in the degree of bilateral symmetrical organization attained before the onset of cleavage.

The existence of a certain organization in the undivided egg anticipating some of the morphological characters of the embryo has been demonstrated and will occupy us on a later occasion (p. 412). There it will be shown that the principal difference between the so-called determinate and indeterminate cleavage types lies in the different degrees of manifest organization present in the egg prior to segmentation. In the extremely determinate types the undivided egg already possesses considerable bilateral and regional organization; consequently their cleavage pattern is as definite and constant as if the cleavage partitions were running on rails. In the indeterminate forms, however, in which the development of inhomogeneities in the egg, such as mentioned above under (1), is considerably delayed, segmentation follows a more haphazard pattern. Even though a certain bilateral organization may be present from the start, further differentiation is so slow that the pattern of cell division becomes more and more irregular, as cleavage progresses. This is the situation which we encounter, for instance, in the egg of the frog, the chick, and the sea urchin.

In conclusion, the question formulated above (p. 198) can be answered as follows: *Segmentation is not an instrument for producing typical disparities and differentiations among blastomeres which could serve as basis for the ensuing formation of the embryo.* Neither a scheme of orderly *nuclear* segregation nor a regular pattern of *cytoplasmic* cleavage can be regarded as fundamental for development. The former does not exist (examples 14 and 15), and the latter is an effect rather than a cause; even in the strictly determinate cleavage types, it is the precocious organization of the cytoplasm that produces the definite cleavage pattern, rather than an orderly cleavage producing organization.

PREFORMED ORGANIZATION

Progress of organization in the absence of cleavage.

Having failed to find the clue to embryonic differentiation in the cleavage pattern, we must turn back once more to the *constitution of the undivided egg.* One is bound to conclude that the fundamental processes responsible for the differentiation of the embryo operate continuously and proceed steadily within the protoplasm of the egg, and are more or less unconcerned with the process of segmentation which happens to involve the same protoplasm at periodic intervals. Cleavage, in a sense, is superimposed upon the progress of intrinsic differentiation of the germ. If this view is correct, one should expect that experimental suppression of cleavage would not suspend the progress of differentiation in the egg. This expectation has been borne out by the following experiments:

Example 18.[1]

When eggs of the worm, Chaetopterus, either fertilized or unfertilized, are exposed for a certain time to sea water to which

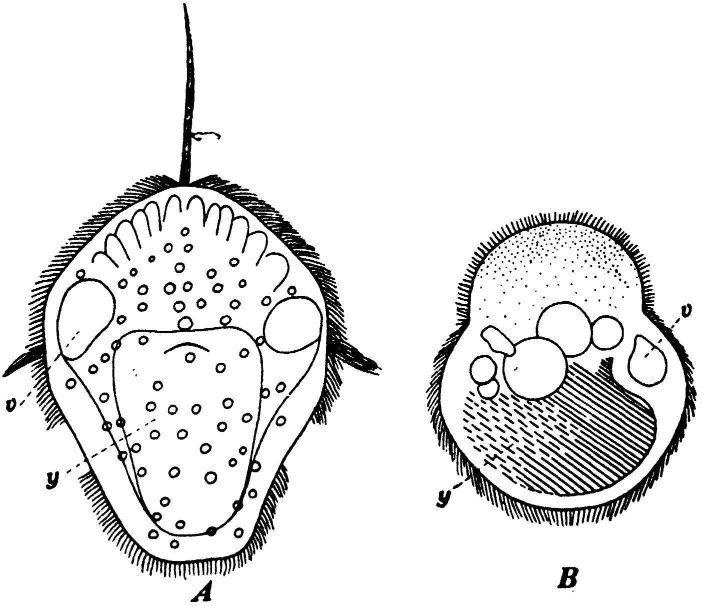

FIG. 38. Differentiation without cleavage in the worm, *Chaetopterus*. (After *Lillie* from *Schleip*)

A, Normal trochosphaere larva.

B, Larva differentiated from activated egg which has remained undivided. Ectoplasm corresponding to ectoderm has covered the surface and developed typical cilia. The yolk (y) representing the entoderm has moved into a position corresponding to that in a normal larva, and the vacuoles (v) have assumed a position roughly at the normal level.

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[1] *F. R. Lillie*, 1902; *J. Brachet*, 1937.

> some KCl has been added and then are returned to ordinary sea water, their protoplasm undergoes marked changes while segmentation fails to occur or remains abortive. The progressive structural changes in the undivided cytoplasm are so similar to corresponding phenomena in the development of a normal larva that they can rightly be called "differentiations" (Fig. 38). The ectoplasm becomes vacuolated like the ectoderm of a normal larva, it develops cilia, and the yolk becomes condensed into a compact mass. Sometimes certain regions of these unsegmented "larvae" can be identified with definite regions of the normal trochosphaere larva. On the other hand, despite this resemblance in the gross lines, the noncellulated bodies never approximate the orderliness of cellulated larvae.

This experiment is instructive in more than one way. It shows, first, that the capacity to develop the specific ground plan of the embryo is present in the egg before segmentation; second, that this capacity can materialize up to a certain point without the intervention of segmentation; third, that unless segmentation does accompany differentiation, the result of such development is abnormal and incomplete.

These points deserve special emphasis. To begin with the last, it shows that segmentation, while in no way responsible for the localization of embryonic parts, still must come in sooner or later if development is to be normal. In other words, we recognize differentiation and segmentation as two series of events independent of each other in a certain measure, but whose *cooperation* is essential for normal development. For the first time we are confronted with a concrete proof of our introductory statement (p. 14) that what we call "development" is in reality a complex affair of many component processes which normally cooperate and interlock so smoothly that their individual contributions to the common result cannot be discerned unless their connection is artificially disrupted by experimental interference. The other points prove that one is justified in considering differentiation and cleavage as collateral processes. This makes the attempt to elucidate organization by studying cleavage look rather futile.

We may, therefore, give isolated consideration to the processes of differentiation in the egg, ignoring their connection with segmentation. Some characters of the undeveloped egg are definitely related to the mitotic mechanism of cleavage, while others are more immediately in the service of differentiation (p. 197). It is these latter characters on which we propose to focus our interest from now on.

Correspondence between the morphology of egg and embryo.

Only very few constant features of the embryo are discernible in the undivided egg. One almost universally found is *polarity*, i.e., a difference in the appearance of the egg along one main axis (p. 189). Morphological and physiological expressions of this axiate pattern were mentioned above. It bears a definite relation to the axial orientation of the later embryo. In many forms, material from around the *animal* pole, if traced into the later embryo, is found somewhere in *anterior* portions of the embryo, while the vegetative materials will be recovered somewhere in the interior and nearer the posterior or ventral parts. Incidentally, whenever in the following mention is made of the fate of certain egg axes in development, these axes should not be visualized as straight geometric lines with an existence of their own. Just as the main axis of a worm is bent when the body coils up; just as our own body axis is twisted when we look behind us; so the egg axis does not remain a straight line during the subsequent stages of development.

Turning to the *disposition of visibly different substances* in the egg cytoplasm, we discover additional characters definitely anticipatory of embryonic organization. As a rule, the materials situated near the animal pole of the egg will give rise to ectoderm (outer covering and nervous system), whereas the parts around the vegetative pole will later be found incorporated in the entoderm (embryonic gut). The fact that localized regions of the egg can be roughly identified with future germ layers, raises the question as to how pertinent this relationship is. Is it mere coincidence that the entoderm develops in the vegetative region where the yolk is concentrated, or is the concentration of the yolk an active factor in determining entodermal differentiation?

Is there prelocalization of embryonic parts?

If one considers that in oligolecithal eggs (i.e., those containing little yolk) the difference in yolk content between vegetative and animal cells is negligible, one cannot very well concede a determining role to the *yolk* itself. There are other distinctions, however, between vegetative and animal portions of an egg besides the different concentration of yolk; these concern the protoplasm proper and might easily have a fundamental bearing on the development of ectoderm and entoderm, respectively. The microscope often fails to reveal the existence of intrinsic differences between animal and vegetative protoplasm, particularly if there is no yolk present to serve as label for the latter. But by the use of special methods, such as vital dyes[1] (see p. 193) or dark field microscope (p. 127), differences can be brought out even in occult cases.

Significant protoplasmic distinctions in an echinoderm egg.

When the egg of a sea-urchin is observed under the dark field microscope, the surfaces of the animal and the vegetative caps appear differently colored;[2] this indicates differences in the colloidal constitution of those areas, and is undoubtedly a reflection of underlying chemical differences. The outline between animal and vegetative parts is quite sharp. One must conclude, therefore, that even in the echinoderm egg, which appears rather homogeneous to ordinary inspection, there is a clear distinction between animal and vegetative protoplasm. Of course, the mere fact that these differences exist, does not yet reveal whether or not they have a bearing on the developmental qualities of ectoderm and entoderm. But there is an experiment which suggests that they have.

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[1] *Spek,* 1934.

[2] *Runnström,* 1928a.

Example 19.

> In a normal sea urchin egg the vegetative material distinguishable by its dark-field color can be traced into the blastula. There it is contained in the cells of the lower hemisphere. These cells are the same which, at the beginning of gastrulation, are invaginated to form the primitive gut of the larva. It has been known for some time that treating the unsegmented eggs of sea urchins with lithium salts greatly modifies the process of gastrulation, in that gastrulae from such eggs possess an oversized entoderm which, being unable to invaginate into the much smaller cap of ectoderm, remains a large extroverted mass. Such forms are called *exogastrulae*[1] (Fig. 55A). Now, when unsegmented eggs were immersed in lithium for a certain length of time and then were examined under the dark-field microscope, it was noted that the characteristic color of the vegetative material had expanded far up into the animal hemisphere for a distance varying with the intensity of the treatment.[2] This demonstrates that *the disproportion between entoderm and ectoderm* typical of the lithium germ and manifesting itself at the time of gastrulation *is preceded by a corresponding disproportion between vegetative and animal materials in the unsegmented egg.*

These results intimate that the qualities of those peculiar substances of characteristic colloid-optical appearance which are located in the surface of the vegetative half of the egg are specifically connected with the formation of entoderm.

There is some prelocalization.

Here we encounter for the first time *a localized cytoplasmic region which can be definitely linked,* even quantitatively, *with a circumscribed embryonic formation.* In connection with the remarks on page 192, it seems significant to note that this area lies in the surface of the egg. One used to refer to egg substances with specific relationships to certain organs of the embryo as "*organ-forming substances.*"[1] This term is rather prejudicial and misleading. All we ought to say is that specific *relationships* exist between certain localized areas of the egg and certain localized formations of the embryo. We thereby avoid the unwarranted implication that the substances contained in those areas actually "form" organs, as a bee forms wax. The term "*organ-forming areas*"[2] seems more appropriate, although "*organ-specific areas*" would be better still.

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[1] *Herbst*, 1893.

[2] *Runnström*, 1928a.

Since the demonstration of organ-specific areas in the egg might easily be misinterpreted as supporting the old idea of *preformation,* attention must be called to some qualifications. First, the vegetative part of the egg is not yet in itself entoderm though it is, in a certain sense, its precursor. Second, the entoderm itself does not remain a homogeneous tissue but continues to differentiate into a variety of structures none of which were represented as corresponding subdivisions within the undeveloped vegetative protoplasm. In other words, the problem of differentiation and localization has not been brought any nearer to its solution by the recognition in the germ of two areas of different qualities, because within each one of these areas the problem arises anew. For the purpose of our present discussion it seems best to record the plain facts, as they are, ignoring for a while their possible bearing on the alternatives of preformation or epigenesis.

If we search for differentiations other than those into animal and vegetative plasms within the undivided echinoderm egg, the yield is rather meager. In many forms no further differentiation can be detected with our present methods. In other eggs, a slightly greater range of diversity is found. In *ascidians* and *amphibians* a third distinctive area has been recognized; it is interposed between the animal and vegetative materials and is specifically connected with the appearance of the third germ layer, the *mesoderm*. This area appears as a sub-equatorial crescent with its greatest width along one meridian and tapering out symmetrically to both sides. It is called "*yellow crescent*" [1] in the ascidians (Fig. 39) and "*gray crescent*" in the amphibians [2] (Fig. 73). It is significant to note that its position is again in the *surface* of the egg.

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[1] The concept of formative substances, originally established by *Sachs* for plants, was extended to animal development by *Wilson,* 1904; *Rabl,* 1906; *Conklin,* 1908.

[2] *His,* 1874.

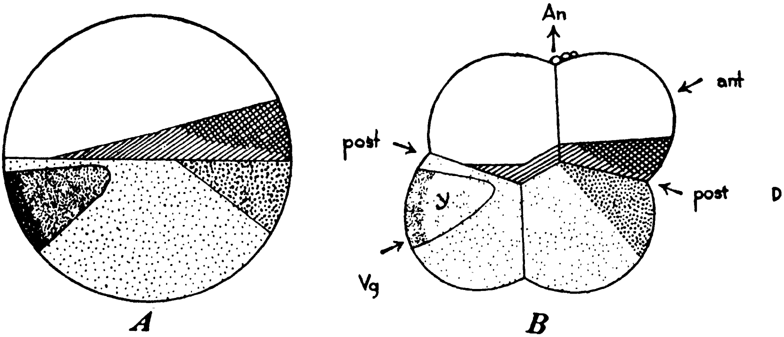

FIG. 39. Topographical chart of organ-specific areas in the ascidian egg (*Styela*). Lateral view. (*Vandebroek*, original) Through the application of vital stain marks the various constituents of the larva could be traced to the differently shaded regions of
A, the fertilized egg;
B, the 8-cell stage.
densely stippled . . . yellow crescent (y); sparsely stippled . . . presumptive entoderm; darkly stippled . . . presumptive notochord; hatched . . . presumptive nervous system; cross-hatched . . . presumptive brain; white . . . ectoderm. An, Animal pole. Vg, Material of the original vegetative pole which has shifted after fertilization.

The gray crescent of amphibians.

The symmetrical arrangement of the crescent imposes bilateral symmetry upon the otherwise rotational structure of the egg. The meridian cutting through the crescent where it is widest and bisecting it geometrically into two symmetrical halves thereby becomes the *plane of symmetry of the egg*. This bilateral-symmetrical disposition of the egg is then carried over into the embryo so that *the plane of bilateral*

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[1] *Conklin*, 1905.
[2] First described by *Roux*, 1888.

symmetry of the undivided egg becomes the later median plane of the embryo.[1] This relationship is much stricter than the one between first cleavage plane and embryonic symmetry (p. 220). In amphibians, if the first or second cleavage plane divides the gray crescent symmetrically, as it does in a majority of cases, then it coincides with the median plane of the embryo. If, however, the first cleavage plane and the plane of symmetry of the gray crescent diverge, the orientation of the embryo follows the latter rather than the former.

The formation of the crescent furnishes a striking example of differentiation in the egg cytoplasm prior to and, therefore, independently of cleavage. In ascidians the crescent is not present as such in the unfertilized egg; at least it is not in its proper location. Only after fertilization has caused widespread movements and redistribution of the egg content, does a certain material which has been recognizable in the unfertilized egg stream into the location and assume the shape of what from then on is the yellow crescent. This happens before cleavage sets in. In amphibians the crescent makes its appearance after the egg has been laid. But whereas in the frog the stimulus of activation is required to initiate the movement of certain egg materials into the area of the gray crescent, in the axolotl, for instance, the movement occurs already in the mature unfertilized egg.[2] In those cases in which the impetus of activation must intervene, it does so again in the entirely unspecific capacity of a release. Its effect perhaps amounts to nothing more subtle than an increase in the mobility of the cytoplasm facilitating the shifting of materials; however, nature and direction of the shifts are determined solely by the constitution of the egg itself.

After the gray crescent has been formed by localized streaming of superficial egg material, the amphibian egg,

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[1] *A. Brachet*, 1905; an even greater extent of prelocalization in the amphibian egg has been suggested by *Pasteels*, 1932.

[2] *Banki*, 1927.

still unsegmented, is found to possess a vague, though fundamental, resemblance to the embryo. This is expressed in the correspondence of axial relationships (p. 225) and in the presence of roughly localized fore-runners of the three germ layers in the form of the animal materials for the ectoderm, the vegetative materials for the entoderm, and the crescent for the chorda and mesoderm. We shall return to this particular case on a later occasion (p. 350). For the moment, it may serve to illustrate the fact that in some forms *there is a segregation of different areas in the cytoplasm* (preferentially at the *surface*) *of the unsegmented egg*, which is evidently a preparation for certain morphogenetic characters of the embryo.

Developmental insignificance of granular inclusions.

Whereas some embryonic characters can be traced back with certainty to distinct localized elements of the unsegmented egg, the converse is not true; that is, *not every discernible differentiation of the egg is of significance for embryonic differentiation.* Pigment granules or other inclusions accumulated in circumscribed regions of many eggs are often wholly irrelevant for the pattern of the embryo, and no significance can be attached to their localization other than that they are indicators of some chemical, physical, or metabolic peculiarity of the surrounding cytoplasm, much as the variegation of a lawn reveals local differences in the composition of the soil. Just as mowing the grass leaves the soil as patchy as it was before, so throwing out the indicator granules from an egg leaves the essential pattern of development unaffected. To test this point, one can actually dislodge the granules from their usual positions by *centrifugation.*

Example 20.[1]

The normal egg of the mollusc, Cumingia, when subjected to centrifugation for a sufficient length of time, appears stratified into four distinct layers. At the centripetal (light) end there is an accumulation of the fatty materials; next to it lies a hyaline clear layer, probably containing the sap and much of the interstitial protoplasm of the cell; then comes the layer of heavier yolk, and finally, at the centrifugal pole, a cap containing the pigment granules which are heaviest. This order is essentially the same for the centrifuged eggs of most forms (see p. 190 and Fig. 32). In some cases the position of the cleavage spindles is influenced by the stratification of the cytoplasm. This is in accordance with the rule that the mechanical conditions of the cell content have a bearing on the orientation of the spindle (p. 206). In the present case, however, cleavage is little affected, and *the centrifuged eggs can develop into typical larvae.* It is important to note that development proceeds while much of the stratified character of the egg is preserved. In other words, it is not necessary that the segregated materials regain their normal distribution after centrifugation has ceased, although a certain measure of rearrangement takes place. At any rate, the *granules remain crowded together* and, when traced into the larva, are found there in some *random location.*

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[1] *Morgan*, 1908.

One must conclude, therefore, that *no localizing or otherwise controlling effect has been exerted by the local segregation of the visible granules.* The same holds true for certain types of eggs in which granules are naturally confined to restricted locations. For instance, in the egg of the worm, Chaetopterus, centrifugation results in the displacement of the localized granular mass, but again, the embryonic pattern remains unaffected.[1] On the other hand, it has also been reported that in some echinoderm eggs the local condensation of granules by centrifugal force does influence the orientation of the embryo.[2] However, this must be regarded as an incidental effect, because fragments of eggs which have been completely deprived of granules still develop satisfactorily.

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[1] *F. R. Lillie*, 1909.

[2] *Runnström*, 1925b.

Example 21.[1]

As described on page 190 (Fig. 32), unfertilized sea urchin eggs, when centrifuged at high speeds (cca. 10.000 × gravity) become stratified according to the specific gravities of their components and finally break apart into a lighter and a heavier half. All granules are contained in the latter. *Either half, however, can develop into a normal larva*, when fertilized. Even quarters of the whole egg, obtained by further centrifugation of the halves (Fig. 32) can develop after fertilization, in spite of the fact that each one contains a different fraction of the egg content.

Centrifuged eggs develop despite disruption.

The centrifugation experiments, in addition to showing that granular inclusions in the egg are of no significance for the localization of embryonic parts, bring out another point very clearly: they prove that a fair degree of development is possible even after an egg has undergone such a violent treatment as the impact of a gravitational force of many thousand times the normal intensity.[2] It must be realized that the effect is not a mere displacement of formed inclusions of second rate importance, but that *the whole matrix of the egg must necessarily suffer, too.* This touches upon the fundamental question as to whether the matrix of the egg contains any *solid framework* serving as a backbone, as it were, for the ensuing embryonic organization. It seems inconceivable that any stable mechanical arrangement could endure the forced separation of the egg content and the convection of granules and other formed particles through the interior without being destroyed. Obviously, only those properties of an egg that survive centrifugation unharmed can be considered essential for development, and a rigid mechanical structure of the interior certainly cannot qualify under these terms. Accordingly, *the idea of a solid framework inside the egg must be abandoned* (see p. 192).

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[1] *E. B. Harvey*, 1932, 1936; the first observations on the development of centrifuged echinoderm eggs were made by *Lyon*, 1906.

[2] *Beams and King* (1937) have described the development of ascaris eggs which had been subjected, prior to cleavage, to a centrifugal force of 400.000 times gravity for 30 minutes.

In this connection it should be considered, however, that centrifugation affects primarily the interior of the egg, whereas probably much less disturbance is created in the surface layer. Possibly the most superficial materials stick so firmly to the membrane that they can retain their typical locations and relations throughout the process of centrifugation. If the crust (cortex) of the egg should prove to be of greater importance in development than the core, as has been suggested (see pp. 193 and 227), then centrifugation experiments would miss the point.

An experimental test of the mosaic concept.

Up to now we have reported circumstantial evidence for the presence in the undeveloped egg of distinct areas which can be linked with specific portions of the embryo. For crucial evidence, however, one must turn to the three critical experimental procedures outlined above (p. 148): (a) *defect,* (b) *isolation,* and (c) *recombination.* Specifically the questions are: (a) Does a local defect in the cytoplasm entail a local defect in the embryo? (b) Does an isolated fragment of cytoplasm give rise to a corresponding embryonic fragment? (c) Does the displacement of cytoplasmic portions result in heterotopy of embryonic organs? Issue (b) is frequently unapproachable for technical reasons (p. 150); the other points, however, have been tested on a wide variety of forms. We choose here as our first example the egg of the *ctenophore.*

Defect experiments in ctenophores.

In the ctenophore egg after fertilization a layer of cortical protoplasm, clearly distinguishable from the central protoplasm, streams down along the surface of the egg, rises again along the forming first cleavage plane and, repeating this behavior during the following divisions, collects gradually at the animal side.[1] In the fourth cleavage step, which leads

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[1] *Spek,* 1926.

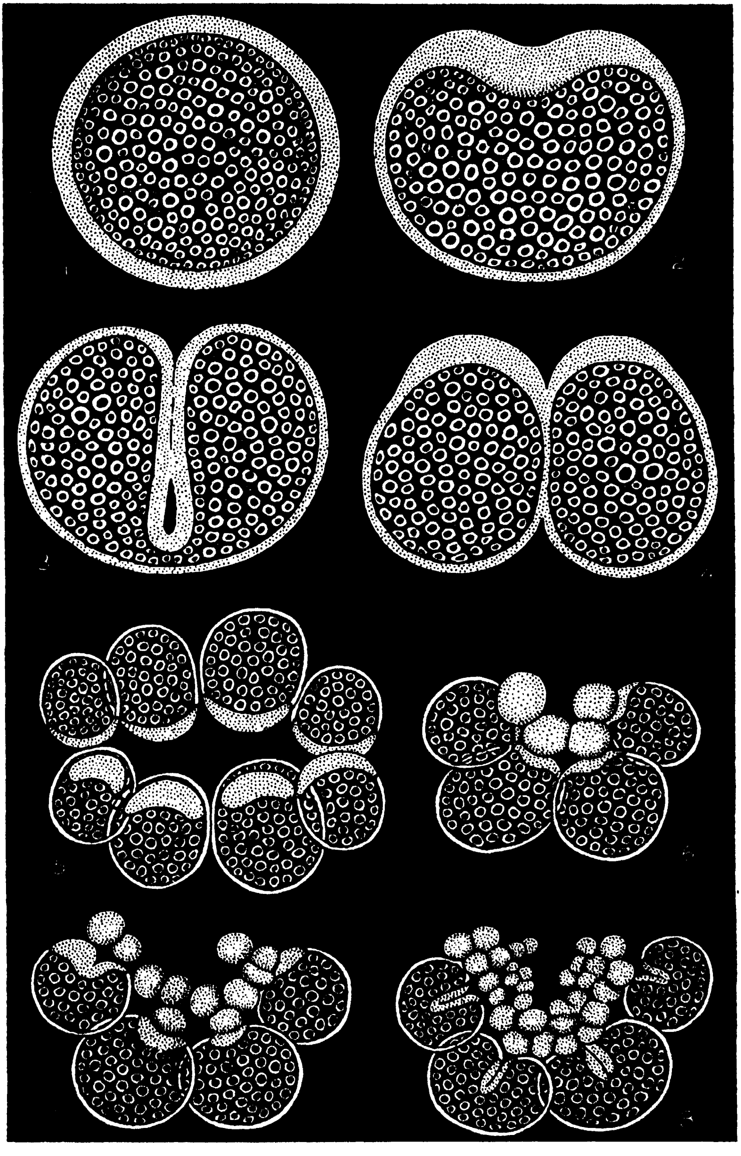

FIG. 40. Redistribution of cortical (micromere-forming) protoplasm during the cleavage of the ctenophore, *Beroë ovata*, as seen in the dark field. (After *Spek*) All views lateral, except 5, which is a polar view.

1–4, First cleavage division. 5, 8-cell stage with accumulation of micromere material near the upper pole. 6, Pinching off of first micromeres; transition from 8-cell to 16-cell stage (only one half of the germ is shown). 7, 8, Continued micromere formation (only one half of germ shown).

from the eight- to the sixteen-cell stage, a full quota of this material is apportioned to the four pairs of micromeres (p. 214) which are budded off on the animal side (Fig. 40). Once collected into the micromeres, this material becomes easily accessible to experimental manipulation.

Example 22.[1]

Defect experiments in which one or more of these micromeres were removed, either alone or in connection with the large blastomeres, proved that the micromeres indeed perform a special function in the formation of the individual. While a normal ctenophore possesses eight rows of comb-like swimming plates, arranged meridionally on the barrel-shaped body, specimens developed from operated eggs show a *deficit* in the number of rows roughly proportional to the loss of micromeres. The blastomeres of the eight-cell stage can easily be separated and raised isolated or in various combinations. The resulting individuals are, on the whole, normal except that they possess only as many rows of swimming plates as there were blastomeres of the eight-cell stage left with the germ (compare example 5). Thus, a larva developing from a single eight-cell blastomere

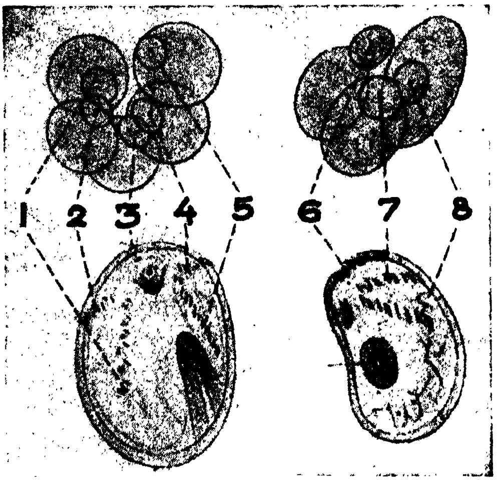

FIG. 41. Early localization of organ formation in ctenophores. (After *Fischel*, 1897) An egg of *Beroë ovata* in the 16-cell stage has been transected into two unequal parts (upper row), one retaining five, and the other three, micromeres. Each fragment develops into a larva (lower row) with just as many rows of swimming plates as it had contained micromeres. Both fragments add up to the normal number of rows, which is eight.

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[1] *Fischel*, 1897, 1898; also *Ziegler*, 1898; *Yatsu*, 1912.

> possesses only one row; a larva developed from three blastomeres, three rows, and so on (Fig. 41). This shows that *each blastomere of the eight-cell stage contains something which is instrumental in the formation of just one line of swimming plates.* An extension of these experiments to the sixteen-cell stage proves that this something is *localized in the animal portion* which at this stage becomes pinched off in the form of *a micromere.* For, if in the sixteen-cell stage the regular arrangement of the micromeres is disturbed by pressure, the rows of swimming plates of the resulting individuals are likewise disarranged.

Ignoring for the time being the fact that the body of the experimental larvae was fairly proportionately built, it seems clear that development has been defective so far as the formation of the swimming plates is concerned. Each micromere of the sixteen-cell stage seems endowed with a definite quantum of factors responsible for the formation of just one line of plates, so that the removal of a certain number of micromeres eliminates the development of a corresponding number of rows of plates. Without knowing the nature of these factors, we can state that they reside in the micromere material. And since this material has been traced back to the cortical protoplasm of the egg, the formative significance of localized cytoplasmic materials seems to be experimentally demonstrated. It will be noted, however, that in this case the location of these materials in the egg bears no immediate relation to the place of their later utilization; they are secondarily swept into their final positions by currents in the egg.

Defect experiments in annelids and molluscs.

The existence of organ-specific regions in the cytoplasm has been demonstrated most convincingly among the eggs of annelids and molluscs. These belong to the most extremely determinate cleavage type, the spiral type. It was reported above (p. 217) that in some eggs of this group the cell which becomes the ancestor of the trunk mesoderm, the so-called entomesoblast, has a traceable history; from earliest cleavage stages on those blastomeres which are in its direct ancestry are distinguished by size and the presence of a peculiar variety of protoplasm, which originally lies near the vegetative pole of the undivided egg and, therefore, is called *polar plasm.*[1] The fact, however, that continuity of substance between this polar plasm and the cells producing the mesoderm has been ascertained, does not yet reveal whether or not there is a causal connection. Defect experiments are at hand to show that there is.

The application of a localized defect (see p. 158) is easier if the egg is already divided into blastomeres. The removal of a whole blastomere allows the defect to be circumscribed more clearly, and the operative procedure is simpler than in the undivided egg. The walls between the blastomeres act like bulkheads. They prevent the contents of the other blastomeres from flowing out when one is destroyed, while local injury to the undivided egg frequently causes the whole egg to collapse. For technical convenience, therefore, most of the original defect experiments were done on segmenting rather than on undivided eggs. It must be borne in mind that a cleaving egg is older than an unsegmented egg and that its condition may have changed sufficiently to yield different results than would be obtained from an undivided egg.[2] With this qualification in mind, we shall now consider a few typical defect experiments.

Example 23.[3]

In some molluscs, e.g., Ilyanassa, the polar plasm bulks so large that it forms during the first cleavage steps a pedunculated protrusion — the so-called *polar lobe,* or "yolk" lobe (although it is practically devoid of yolk) —; the lobe remains attached to one blastomere into which it is periodically withdrawn during the intervals between divisions. While mitosis is under way, it is easy to amputate this lobe from the main body of the blastomere. Eggs thus deprived of the polar lobe can develop but fail to form an entomesoblast, and the resulting larvae are conspicuous by the complete *lack of mesoderm.*

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[1] Comprehensively reviewed by *Schleip* (1929).

[2] The importance of this point will become obvious later, page 406.

[3] *Crampton,* 1896.

Thus, there can be no doubt but that *the polar plasm of the undivided egg contains some factor essential for the formation of the later mesoderm.*

Example 24.[1]

The egg of the mollusc, Dentalium, follows the typical spiral cleavage type (p. 211). If the first two blastomeres are separated, and reared apart, both continue to cleave as if they were still in contact with each other. Each divides into two macromeres, then micromeres are produced in a sequence of steps typically corresponding to the half of the egg represented by the isolated fragment. In the half containing the polar lobe the entomesoblast and its derivatives, the teloblasts, appear in due time, while these cells are missing in the other half which was devoid of polar plasm. In other words, each half in isolation behaves in the same manner as it would have behaved had it been left undisturbed and in conjunction with the other half. The *internal organization of both fragments shows specific defects* which are much more serious in the half lacking the polar plasm than in the half possessing it. In the former, the mesoderm, foot, shell and mantle rudiments of the larva are absent. Just as in the preceding example, the lack of these organs can be ascribed directly to the absence of their formative material, because the suppressed parts are precisely the ones known to arise from cells containing polar plasm. But in addition to the absence of these organs, the lobeless larvae show another defect which cannot be traced to lack of specific building material: the *apical tuft* which should have developed at the animal pole is also missing.

One must conclude, therefore, that the polar plasm, besides producing mesodermal organs, also exerts some control over organs arising in the animal region of the germ, although these lie at the opposite pole and apparently are not descended from the cell line connected with the lobe. That this control is effected at a comparatively early stage, is proved by the fact that if the polar lobe is removed in the four-cell stage instead of in the two-cell stage, the resulting larva, while lacking the mesodermal organs, does possess an apical tuft. By that time the polar plasm obviously has already exerted its critical influence upon the apical material. This point deserves emphasis because it demonstrates that *specific interactions among parts* during post-fertilization stages occur even in those forms whose development is commonly thought of as rigidly predetermined. A more thorough discussion of this point will be found in a later section (p. 406).

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[1] *Wilson,* 1904.

These results stress the formative role of the polar plasm and show, as it seems, incontrovertibly that *tangible forerunners of certain definite embryonic formations can be detected in the cytoplasm of these eggs as early as at the beginning of segmentation.*

Example 25.[1]

Defect experiments in ascidians.

> In the egg of the ascidian, *Styela*, the pattern of cleavage conforms with the strictly bilateral-symmetrical organization of the egg in which the appearance of the yellow crescent (see p. 229) serves as an early indicator of the fundamental pattern of the embryo (Fig. 39). Owing to this fact, it is possible to state for any pair of cells of the four-cell stage whether they lie dorsally, ventrally, to the left, or to the right, with regard to the axes of the future embryo. If a lateral half of the egg in the two- or four-cell stage is paralyzed by mechanical injury, the other half continues to develop. The developing half cleaves according to a pattern essentially like the one followed ordinarily by the same half in a nonmutilated germ. Differentiation in the surviving half also proceeds in the same way as if the other

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[1] *Conklin,* 1905; earlier experiments with isolated blastomeres of ascidian eggs had been performed by *Chabry,* 1887.

half were developing alongside. Thus, an individual is formed which has essentially the organization and composition of a *half* larva; it contains half the normal cell number, paired organs are represented only by a single unit of regular size, while organs along the midline which normally would be composed of contributions from both sides, are reduced in size (Fig. 42). Some secondary rearrangements among the parts occur as a result of the abnormal mechanical conditions, but they are not so comprehensive as to obscure the primary *halfness* of these larvae. If dorsal or ventral blastomeres are isolated instead of lateral ones, it is not so easy to recognize their products as the corresponding halves of a normal embryo, because many secondary cell shifts which normally take place between anterior

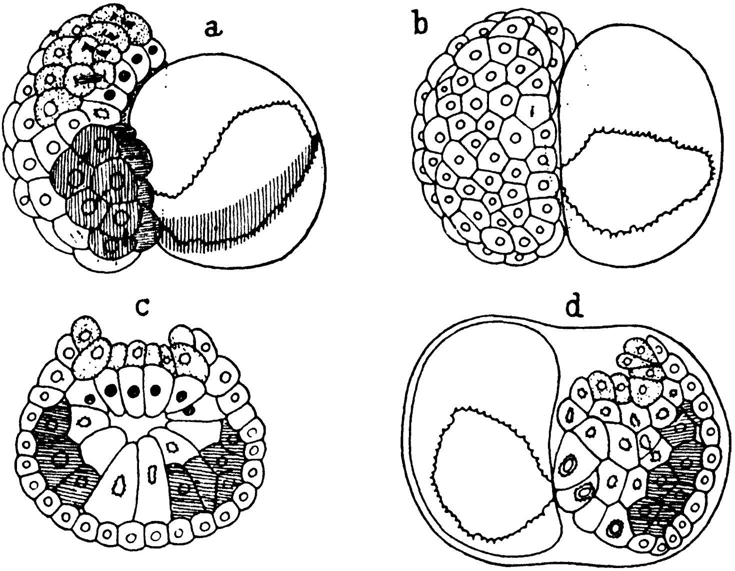

FIG. 42. Mosaic development in the ascidian, *Cynthia* (*Styela*) *partita.* (After *Conklin* from *Schleip*) After injury to one of the first two blastomeres, the surviving blastomere developed into a half embryo.

a, Dorsal view of half gastrula, adhering to the dead blastomere. Number and disposition of the mesenchyme, muscle, neural and chorda cells correspond very nearly to those of a lateral half of a normal embryo.
b, Ventral view of same germ showing only ectoderm cells.
c, Cross section through normal embryo.
d, Cross section through a half embryo.

and posterior parts are missing in the abnormal forms. Nevertheless, it has become clear that embryonic parts developed from isolated anterior or posterior halves of the egg also represent grossly the same portions which arise from these egg regions in normal development.

The mosaic theory scores a point.

Two facts stand out clearly from all these experiments. First, each fragment of the egg continues after isolation to *cleave* according to the *partial* pattern which it would have followed under normal circumstances. Secondly, *differentiation* and *localization* also proceed, at least in principle, as if the separated fragments still formed part of the original whole germ. These results cannot fail to give the impression that every part of the embryo has a localized, pre-assigned precursor in the cytoplasm of the early egg; that the organization of the embryo can be projected back upon the egg, part for part; and that development consists merely of the transformation of those pre-localized inexplicit differences in the egg into the manifest differentiations of the embryo, carried out by each part in its own power. While examples 22 to 24 only point toward some localized portion of the cytoplasm as having a specific embryogenetic assignment, example 25 seems to demonstrate an even more detailed *pre-localization of embryonic parts in the egg*, a result strongly suggestive of the tenets of the so-called mosaic conception of development.

Summing up the results of the defect experiments 22 to 25, we can state that eggs of these particular groups can continue to develop after a certain amount of their substance has been removed, but give rise to embryos with definite structural defects. The defects are either narrowly circumscribed (for instance, the absence of a row of swimming plates in the ctenophore) or more general (e.g., absence of the whole mesoderm, as in example 23). Since in the latter case parts are involved whose presence would be indispensable for the later course of embryogenesis, development of defective individuals cannot proceed beyond the critical stage at which the missing parts would assume importance. The significant thing to note is that development can go even this far in spite of the defect, and that in doing so, it follows essentially the same lines along which it would have proceeded had the germ been left unharmed. This patent disregard of parts of the germ for what happens, or fails to happen, in the other parts certainly contradicts the assumption of a unitary control of development through which an active inter-relationship would be entertained among the different parts, subordinating the behavior of all to a collective goal: the formation of a normal individual.

It will be shown below that the results of examples 22 to 25 are by no means amenable to outright generalization; but in these specific examples, at any rate, one does obtain the impression that development of the whole germ is simply the sum total of so and so many independent local processes going on in various parts of the egg concurrently, and that the effect which the suppression of one or more of these partial processes has upon the end result can be calculated by simple subtraction. Under this impression the notion has arisen that *development is mosaic work* in which numerous individual areas of the germ, endowed from the beginning with specific local assignments would grow and mature with little or no mutual interference. Just as the single micromere of the ctenophore egg was thought to contain a definite assignment to produce a single line of swimming plates, so the other parts of the organism were conceived as represented in discrete portions of the germ. *Mosaic theory* in this guise is essentially a re-edition of the old *preformationist theory.*

The implications of the mosaic concept.

Seemingly this theory is supported by defect experiments of the type outlined above. So long as every part of the germ continues a straight course toward its normal limited fate, no matter whether or not the other parts join, there seems to be little place for the assumption that the fate of the whole germ depends on specific cooperation and interaction among its various constituent parts aside from that commonly found among independent and equivalent neighbors pursuing their individual jobs in mutual tolerance and non-interference. Furthermore, inasmuch as every part continues to develop along the same stereotyped lines, with no regard as to whether or not this is beneficial to the community of parts, it seems logical to assume that the parts cannot do otherwise. In other words, each part seems to have a prescribed single course to which it is bound; what it *does* along this one course comprises all that it ever *can do.* This state of affairs is customarily expressed in the biological equation: *prospective potency* equals *prospective fate.*[1]

To explain these terms briefly: *prospective fate* refers to the lineage of each part of the egg through its cell descendants into a definite portion of the adult organism. The methods used to study prospective or "presumptive" fate have been outlined in Part Two, and the general results of such tracing have been illustrated in Part One. All this refers to what *actually* happens in normal undisturbed development. *Prospective potency,* on the other hand, means the full range of developmental performances of which a given part of the germ is *capable* under any conceivable circumstances, natural and experimental.

Self-differentiation.

Equating prospective potency with prospective fate, means to proclaim absolute rigidity in development and to blame the fact that major disturbances are not repaired (examples 22 to 25) on the single-tracked behavior of the elementary parts. It would be of little avail to the developing ctenophore to perceive the damage caused by the removal of some micromeres if it could not thereafter deflect the remaining materials from their original courses and make them substitute for the removed portion. The negative statement, that parts fail to take cues from one another as to what their individual procedures in the total developmental process is to be, implies, of course, a positive corollary, namely, that each part must have a very definite instruction from the beginning directing it toward its final partial goal. This capacity of a part of a developing system to pursue a specific course, the character of which is entirely defined by properties intrinsic to the part itself, has been called *self-differentiating capacity.*[1]

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[1] These terms originally introduced by *Driesch* have been generally adopted.

The implications of the term self-differentiation are frequently misunderstood. The emphasis lies on the fact that the *specific character* of the transformations of the part is determined from within rather than from without. This does not imply, however, that the part would under any circumstances be able to proceed with the realization of this intrinsic tendency. We may simply point to the remarks made in Part Two concerning the specific versus the unspecific requirements of fragments of an organism when kept in isolation. It goes without saying that the actual manifestation of self-differentiating power is conditional on adequate necessities of an unspecific character, such as proper milieu, temperature, oxygen, nutrition. Consequently, self-differentiating capacity is best defined as the ability of parts to develop, under conditions favorable to development, into something *specifically different* from what would be produced by some other parts under otherwise identical conditions.

Is all development mosaic work?

Now, indeed, if every part of an egg were endowed with self-differentiating power, then a developing organism would really be but a bundle of independently developing parts united through mechanical coherence. Whether this would make it any easier to *understand* development is a different question. We are concerning ourselves here only with the problem of whether it is a fact or not. The experiments outlined above, pertaining to ctenophores, annelids, and molluscs encourage the view that it is. Not even the centrifugation experiments (example 20) can weaken the evidence because, as we have said, a surface mosaic might have remained unperturbed.

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[1] The term *self-differentiation* was coined by *Roux*, 1881.

Let us, therefore, turn to another class of eggs, those of echinoderms and vertebrates, and see whether they exhibit the same kind of mosaic behavior. Curiously enough, we find it there only if we experiment with sufficiently late stages.

The development of the sea urchin.

Briefly, the embryonic history of a sea urchin egg is as follows (Fig. 43):

The first two segmentations (*a*, *b*) occur along meridional planes and produce four equal blastomeres. The third step (*c*) is an equatorial division separating four upper, animal, blastomeres from four lower, vegetative, blastomeres. In the fourth cleavage step (*d*) the four animal cells are divided meridionally into eight equal cells, so-called *mesomeres*, while the four vegetative cells undergo a very unequal division, parallel to the equator, into four large upper cells, the *macromeres*, and four small cells around the former vegetative pole, the *micromeres* (see p. 214). In the fifth step (*e*) the mesomeres are divided horizontally and the macromeres vertically. The thirty-two-cell stage is, therefore, composed as follows, from the animal to the vegetative pole: (1) a plate of cells (mes_1) formed by the upper halves of the former mesomeres surrounding the former animal pole; (2) the lower, near-equatorial daughter cells (mes_2) of the mesomeres; (3) the divided macromeres; (4) the micromeres. At the next (sixth) cleavage step the eight macromeres divide horizontally into an upper (mac_1) and a lower (mac_2) group. The fate of these various cell groups has been traced through the blastula and gastrula into the later larva. The results are illustrated in Fig. 44. In the blastula all the cells form a single layer in the surface. Then certain cells around the vegetative pole immigrate into the interior of the blastocœle. These cells, called primary mesenchyme (*p*), are direct descendants of the micromeres and are destined to form the skeleton of the pluteus. Next, gastrulation occurs by the invagination of a large part of the lower surface of the germ largely derived from mac_2; the edge of the invaginating material (entoderm) coincides roughly

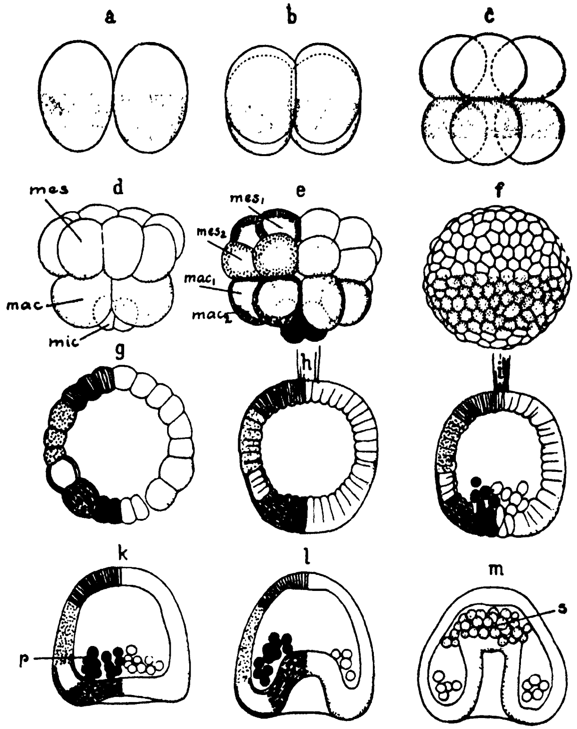

FIG. 43. Cleavage and gastrulation in the sea urchin, *Paracentrotus lividus*. (After *Boveri* from *Schleip*, with additions)

a–f, lateral views of: *a*, 2-cell stage. *b*, 4-cell stage. *c*, 8-cell stage. *d*, 16-cell stage, consisting of 8 mesomeres (mes), 4 macromeres (mac), and 4 micromeres (mic). *e*, 32-cell stage. *f*, blastula. The gray band in the vegetative half represents a ring of orange pigment peculiar to this species of sea urchins.

g–m, meridional sections through germs from the blastula to the gastrula stage. *g*, early blastula. *h*, blastula with apical tuft. *i*, immigration of primary mesenchyme (skeletoblasts) into blastocœle. *k*, beginning invagination of entoderm (p, primary mesenchyme). *l*, early gastrula. *m*, gastrula producing secondary mesenchyme (s).

The fate of the different levels of blastomeres from the 32-cell stage (*e*) through gastrulation is indicated by distinctive markings in the left halves of the germs. For continuation of the sequence, see Fig. 44.

with the boundary between the derivatives of the lower (mac_2) and the upper (mac_1) macromeres. The remaining cells, derived from mes_1, mes_2, and mac_1, occupy the outer surface of the gastrula and form ectoderm. Specific formations of the *ectoderm* in the later larva are an apical tuft (a temporary formation); ciliary bands girding the oral region of the larva; and the stomodaeum. The tuft marks the original site of the animal pole, the stomodaeum the ventral side. The invaginated *entoderm*, first a blind sac (primitive gut, archenteron), later bends ventrad and at its tip breaks through into the stomodaeum. The *mesenchyme* produces the skeletal rods.

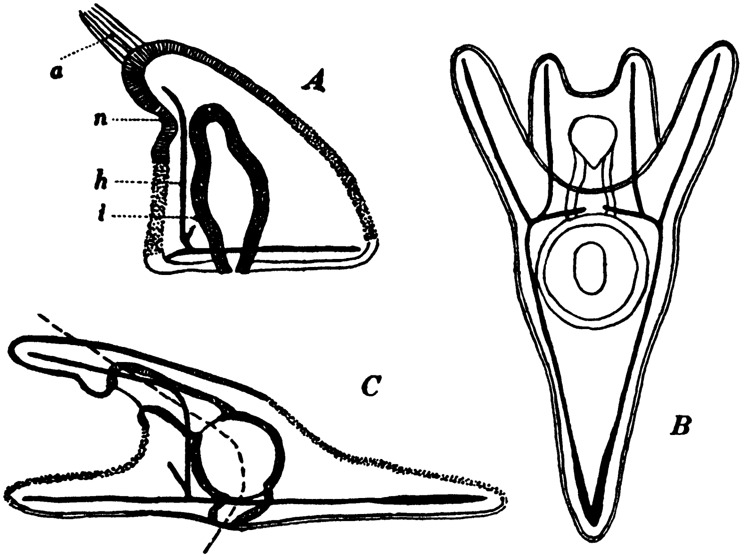

FIG. 44. Later stages of sea urchin development. (From *Hörstadius*, 1935)
A, End of gastrula stage. Lateral view. Dorsoventrality clearly expressed. *a*, Apical tuft. *n*, Stomodaeum. *h*, Skeleton. *i*, Gut.
B, Pluteus, ventral view.
C, Pluteus, lateral view. Stomodaeum has broken through into gut. Dotted line indicates position of original egg axis.
The various regions of the larva have been marked in the same manner as their original sources in the germ, Fig. 43 *g–l*. × 240.

This account indicates the prospective fate of the different portions of the cleaving egg. Whether these portions possess corresponding self-differentiating powers can only be determined by defect experiments.

Example 26.[1]

Experiments suggesting mosaic features of the sea urchin egg.

Sea urchin eggs of the eight-cell stage were divided along the third, i.e., equatorial, cleavage plane into an *animal* and a *veg-*

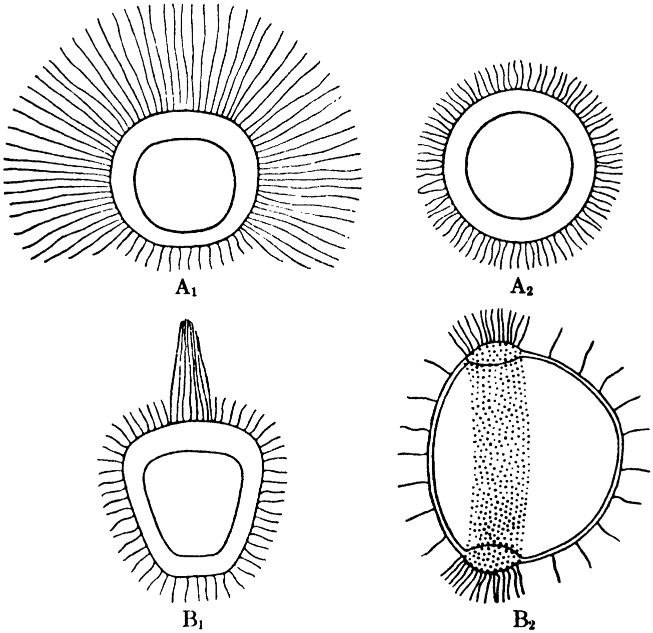

FIG. 45. Differentiation of isolated animal halves of the sea urchin egg. (From *Hörstadius*, 1935)

A_1, Extreme type: blastula almost completely covered with long cilia. × 190.

A_2, Later and final stage of A_1. No differentiation. × 200.

B_1, Less extreme type: long cilia confined to the apical region. × 190.

B_2, Later stage of B_1: a ciliated band resembling that of a normal larva has formed. × 210.

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[1] *Hörstadius*, 1928, 1935. — Many of the pioneering experiments on the sea urchin egg performed in the early days of Experimental Embryology by *Driesch*, *Morgan*, *Boveri*, etc., have been repeated and amplified in more recent years, particularly by *Runnström*, *v. Ubisch*, and *Hörstadius*. We shall base our discussion largely on these recent studies, because they have cleared up several points that had remained uncertain from the earlier work.

etative half. The prospective fate of the former would have been the formation of ectoderm with apical tuft, ciliary bands, and stomodaeum; while the vegetative half would have produced invagination, gut, skeleton, and a small portion of the ectodermal covering. When separated from each other by an equatorial cut, the two halves continue to develop with the following results. The *vegetative half* invaginates, produces skeleton, gut, and an ectodermal covering, but is deficient in regard to the organs which would normally arise in the animal half, i.e., ciliary tuft, stomodaeum and ciliary bands. The isolated *animal half*, conversely, produces the apical tuft, a stomodaeum and a ciliary band, but, on the other hand, fails to gastrulate, neither archenteron nor skeleton is formed, and morphogenesis does not go beyond a blastula-like stage. There are individual variations in the extent of these deficiencies within a range illustrated in Figures 45, 46. It can be seen that the gut of isolated vegetative halves is frequently excessively de-

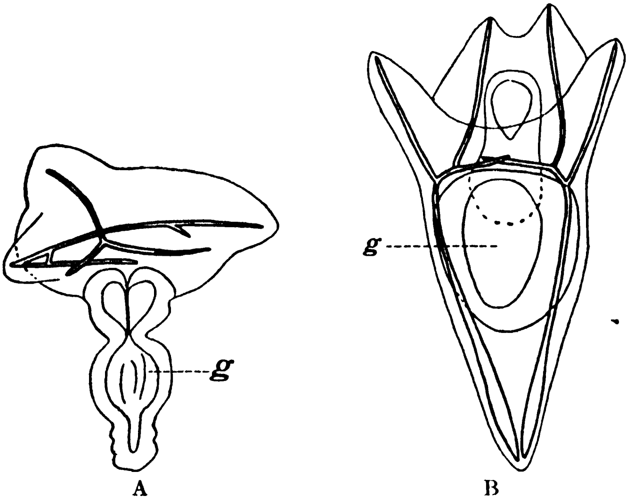

FIG. 46. Differentiation of isolated vegetative halves of the sea urchin egg. (From *Hörstadius*, 1935)

A, Extreme type: evaginated oversized gut (*g*) (exogastrula); animal parts absent. × 260.

B, Less extreme type: almost normal pluteus with somewhat oversized gut (compare with normal pluteus, Fig. 44B). × 260.

> veloped. Isolated animal halves, in turn, show considerable latitude in the expansion of the tuft. Always centering around the original animal pole, it sometimes covers practically the whole surface, and in this case stomodaeum and ciliary bands are suppressed; but even when it is more restricted, it far exceeds normal proportions.

One can see that each isolated half produces approximately its normal quota of organs as indicated by its prospective fate. For instance, the intestinal passage of a normal larva forms by the perforation of the blind gut into the blind stomodaeum, just as a tunnel is drilled from both ends. In isolation, each half of the germ forms its own section of the tunnel. In typical cases both remain blind. Thus, one gets the impression that if an animal half and a vegetative half were reared apart and then recombined at some advanced stage of development, they would add up to a single larva fairly normal in equipment, if not in shape and proportions. This result, therefore, is essentially not different from that obtained by separating animal from vegetative blastomeres in the ascidian egg, and is suggestive of pure self-differentiation of parts.

Self-differentiation of amphibian germ fragments.

In order to obtain in amphibians — and the same holds for birds — evidence of self-differentiation as striking as that described in the preceding examples, one must turn to slightly older stages. Such germs, when bisected, furnish a beautiful demonstration of piecemeal development.

Example 27.[1]

> When the animal portion of an amphibian neurula, which corresponds to the later head region of the embryo, is excised and reared in isolation, it forms a practically *complete, proportionate, and well differentiated head* with no trunk attached (Fig. 47A). It has bilateral symmetry, contains the appropriate brain portions, two eyes with lenses and optic nerves, nostrils,

[1] *Holtfreter*, 1931.

oral cavity, skull, jaws, and muscles. If, on the other hand, a postero-ventral portion of the neurula, approximately complementary to the former fragment, is isolated, it gives rise to *a headless trunk portion* containing axial skeleton, spinal cord, musculature, gut, pronephros, tail, the typical fins, pigment in typical arrangement, and even limbs in the proper sites (Fig. 47C).

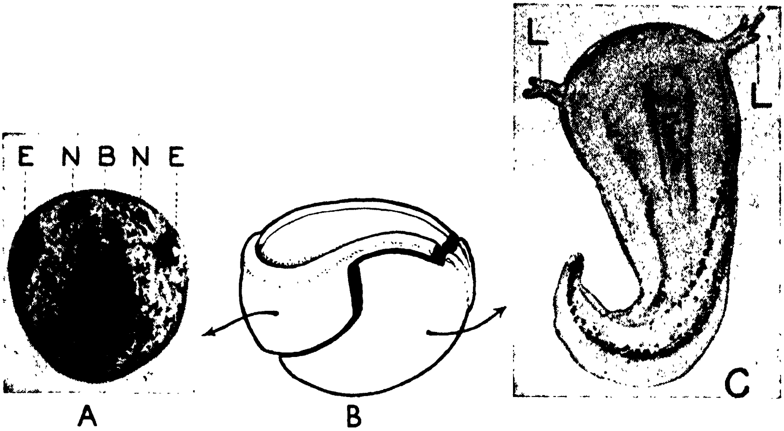

FIG. 47. Self-differentiation of the isolated anterior and posterior halves of a urodele germ. (A and C from *Holtfreter*, 1931) A neurula was divided as illustrated in B, and both fragments were raised separately. A and C show the results 25 days after the operation: a head fragment and a trunk fragment, respectively.

E, eye. N, nose. B, brain. L, fore limb. × 11.

The two complementary fractions of the germ, allowed to proceed with their development independently, have produced two complementary regions of the body; piecing them together in our imagination, we see that they add up roughly to the effigy of a whole animal. This is plainly self-differentiation.[1] Even much smaller fragments of a forming body can self-differentiate. In fact, one can demonstrate the self-differentiating capacity of practically every major organ of an amphibian or bird, provided one selects the proper stage.

[1] Compare also the composite larvae, Figure 28, page 163, produced by the self-differentiation of embryonic fragments grafted together.

Example 28.

Self-differentiation of eye.

When the lateral bulge of the brain wall which forms the rudiment of the eye is explanted (see Fig. 11A), it continues to develop[1] and gives rise to an *eye of a fairly high degree of histological differentiation,* with pigmented tapetum and stratified retina.[2]

Example 29.

Self-differentiation of heart.

In the tail-bud stage of an amphibian, the prospective material of the heart lies in the ventral mesoderm of the anterior trunk region (Fig. 66). If this material is explanted, it forms within a few days a structure which can be identified both morphologically and physiologically as a *heart,*[3] consisting of a tube with the typical symmetrical twist of a heart, subdivided into four portions — sinus, auricle, ventricle, and arterial bulb — and, most significantly, exhibiting *rhythmical pulsations.* Even functionally, therefore, it has differentiated the typical characters of a heart.

Example 30.

Self-differentiation of limb.

The first indication of the appearance of limbs is a thickening of the body wall (Fig. 10). This so-called limb bud is filled with a mass of cells of very homogeneous appearance. When a chick limb bud is explanted into a nutrient culture medium (p. 154) it not only *grows in length* and assumes the *normal shape and proportions of a limb*[4] with fingers, but the skeleton in its interior develops almost normally, and even the joints form in their typical location (Fig. 48). This, incidentally, is conclusive proof that function, in the sense of actual movement, is no factor in the primordial development of joints (see p. 445).

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[1] *Filatow,* 1926; *Perri,* 1934.

[2] *Strangeways* and *Fell,* 1926; *Dorris,* 1938.

[3] *Stöhr,* 1924; *Ekman,* 1924.

[4] *Fell* and *Canti,* 1934.

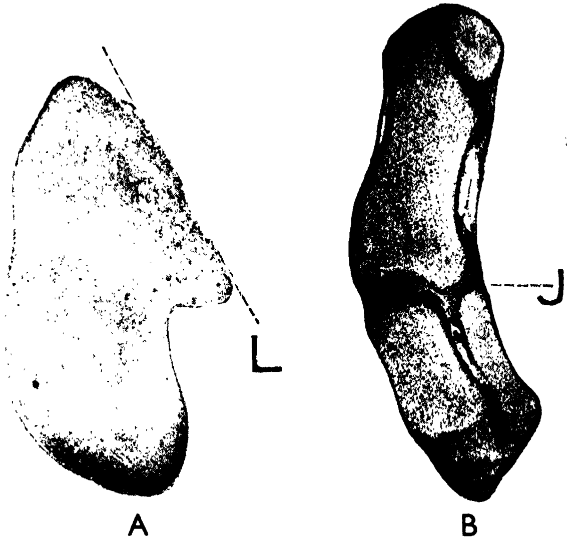

FIG. 48. Self-differentiation of the explanted limb bud of a chick embryo. (From *Fell and Canti*) × 30.

A, Amputated limb bud of embryo (4 days incubation) at the time of explantation into blood plasma (see p. 154). L marks the level of ablation. The coming differentiation of the skeleton is faintly indicated by a Y-shaped condensation of the cells in the core of the bud.

B, Limb fragment after 4 days of cultivation in vitro. A typical cartilaginous skeleton has differentiated. A knee joint (J) has formed in the absence of all movement.

The mosaic of organ rudiments.

The list of examples of self-differentiation of isolated germ fragments could be considerably extended (Fig. 49). Their net result can be expressed as follows: Once the primordium of an organ has become *recognizable* as a fold (example 28), or a local aggregation of cells (example 30), or a thickening of a germ layer, or in some similar form, *its prospective fate and prospective potency can be equated,* even in the "indeterminate" forms. The same fact holds true for a certain period preceding the stage at which the organ rudiment becomes clearly distinguishable. A fragment of mesoderm of the future heart region (example 29) can form a heart even though it was indistinguishable[1] from any ordinary mesoderm at the time when it was explanted. We can, therefore, state that *the area in which an organ primordium is about to make its appearance is endowed with some degree of self-differentiating power.*

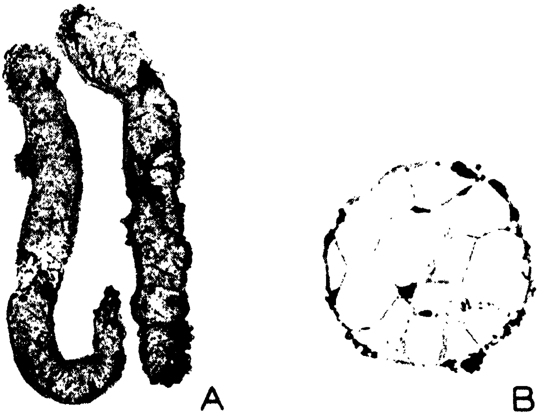

FIG. 49. Self-differentiation of explanted amphibian notochord. (From *Holtfreter*, 1931) The notochordal primordium was excised from a late neurula and raised in salt solution for 11 days. The fragments have assumed characteristic cylindrical shape (A), and cross sections (B) show histological structure typical of notochord. A, × 22; B, × 125.

It must be understood, however, that in all these cases self-differentiating capacity is judged by some specific features of the developed organ, such as the arrangement and histological differentiation of the cells. In many other respects development in isolation differs quite markedly from development inside the organism (see also p. 360). Morphogenesis (p. 68) suffers most. Gross deformations and aberrations in size and proportions are quite common. Furthermore, self-differentiated organs lack the usual contributions from the surroundings, such as nerves, blood, some muscles, ducts. Consequently, organs reared apart from the organism, although they can usually be identified, show various imperfections.[1] Save for this qualification, however, self-differentiating power is an attribute of the majority of *organ rudiments* of vertebrates hitherto tested.

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[1] The term "indistinguishable" in this connection refers merely to *visible* morphological distinctions.

Just as the organ rudiment continues after isolation from the rest of the organism to develop into a definite part, so the residual portion of the germ also self-differentiates and produces a body showing *defects* which, on the whole, correspond to the experimental loss. Thus, after removal of an eye rudiment (example 28) the embryo remains eyeless [2] on the side of the operation (Fig. 69), and the complete extirpation of a limb bud may lead to the permanent absence of the limb,[3] save in the case of secondary regeneration, which will be considered on a later occasion (p. 458 f.).

The mosaic condition is not primordial.

These facts leave no doubt but that an amphibian germ of the *neurula* stage is parceled out into organ-specific districts each of which is capable of effecting a remarkable degree of self-differentiation in complete isolation. Consequently, in the point of mosaic character, an amphibian neurula measures up to an early segmentation stage of an ascidian, mollusc, or annelid. But as soon as we turn from the neurula to much younger stages and test portions of them for self-differentiating capacity, we find little or even none at all. This fact will be considered more fully below. It cautions us against regarding the mosaic condition of the late amphibian germ as a primordial character of the egg. Similarly, in an echinoderm egg, no finer localization of individual parts has been ascertained prior to gastrulation than the crude subdivision into an animal and a vegetative portion reported above (example 26). *We must, therefore, make a clear distinction between results obtained in young germs and those obtained in older ones.*

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[1] Most of the remarks concerning self-differentiation in explants hold likewise true for interplants (chorio-allantoic grafts), see later, page 395.

[2] *Spemann*, 1903, 1912; *Lewis*, 1904, 1905.

[3] *Harrison*, 1918.

PROGRESSING ORGANIZATION

REGULATIVE DEVELOPMENT

Pre-mosaic stages.

Both in echinoderms and amphibians, *early* stages are remarkable for the *indefiniteness* of the developmental future of individual cells. That is to say, if we isolate a group of cells from a sufficiently young germ, we find, first, that they have no tendency to self-differentiate in accordance with their normal presumptive fate, as parts of a mosaic would do, and, second, that the same cells, when subjected to a variety of conditions, can develop along a variety of different lines. The actual fate of a given part in a particular specimen is, of course, definite. But the same part in another specimen can develop in an entirely different direction, and if one pools the information collected from all individual germs ever observed, under normal and experimental conditions, one realizes that there is at first a wide range of opportunities open to the various parts of an egg. Consequently, for these systems, *prospective potency is much wider than prospective fate.* Since, as we have said before, every egg behaves eventually as a mosaic, the preceding stages of less restricted potency may appropriately be called *pre-mosaic* stages.

If we now compare a ctenophore egg of the sixteen-cell stage, as discussed before (example 22), with an echinoderm egg of the sixteen-cell stage, we find the former in a *mosaic*, the latter in a *pre-mosaic* condition. This is clearly demonstrated by *defect* experiments. While in the former case the removal of the micromeres leads to permanent defects, a similar interference with the latter form has no such serious consequences. Although micromeres in ctenophores and in sea urchins are in no way homologous (see p. 214), both have in common a well circumscribed destination in the normal course of development. In the ctenophores they produce the swimming plates, and in the sea urchin they produce the primary mesenchyme and through this the skeleton of the larva (see Figs. 43, 44).

Example 31.[1]

A germ defect that leaves no trace.

The four micromeres of the sea-urchin egg keep on dividing and just before the onset of gastrulation migrate into the blastocœle. By this time they number about fifty. They arrange themselves in two clusters symmetrically with regard to the median plane and soon start their specific performance, the production of the skeleton. It has been revealed by vital staining that normally no other cells but the derivatives of micromeres take part in the formation of the skeleton. If, however, the micromeres are experimentally removed in the sixteen-cell stage, the remaining germ still develops into a perfectly *typical larva possessing a normal skeleton.* This substitute skeleton has been produced by cells that ordinarily form part of the gut. Obviously some of the *presumptive entodermal cells have been diverted from their original course and have vicariously taken over the function of forming skeleton.*

This result proves two things: first, that the substituting cells, although presumptively entodermal, cannot have been definitively preoccupied with the differentiation of gut, or else they would not have been able to switch to skeleton; and, secondly, that some factor must have made them assume the task of precisely those parts which had been eliminated. To be sure, in the present case not much rerouting was necessary to make up for the experimental loss; some cells had to be withdrawn from an otherwise similar group and directed to make skeleton, while the rest of the germ proceeded as usual. However, there are other cases in which the experimental interference necessitates a much more extensive reorganization of tasks within the germ if normal development is to ensue. Such is the case especially after *median transection of an egg.*

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[1] *Hörstadius*, 1935.

A meridional half of a sea urchin germ produces a whole larva.

Whereas equatorial transection of a sea urchin egg, as reported above, divides the germ into an animal and a vegetative half of apparently distinctly different developmental capacities, the halves obtained by dividing an egg meridionally behave identically. Each *half*, instead of producing the corresponding lateral half of an embryo, as has been the case in the mosaic egg of Styela (example 25), develops into a *normal, complete, and proportionate,* though somewhat *undersized, embryo.*[1] By following the various parts of a halved egg into their later destinations, one can easily recognize the great discrepancy between their actual fate and the course which we know they would have taken if the egg had been left undisturbed.

Example 32.

Figure 50 illustrates how the materials of a blastula are employed during the subsequent gastrulation stages, in a normal germ (A, B), and in a half germ which has been split off by a meridional cut (C, D, E). The result is essentially the same whether the splitting is executed in early cleavage stages or as late as in the blastula stage. For convenience, however, we shall base our discussion on the latter case. For the sake of further simplification, let us focus on just two major developments, that of the ciliary tuft as a representative of the *animal* differentiations, and that of the primitive gut as the *vegetative* representative. In an undisturbed germ these two differentiations occur at almost exactly opposite poles of the blastula. In the diagram (Fig. 50) the original poles of the egg are marked, the animal pole by a double cross (⌗) and the vegetative pole by a double ring (◎). The latter symbol at the same time designates the point which will be farthest advanced in the entoderm invagination during gastrulation, i.e., the future apex of the gut. This region is later to become the source of the secondary mesenchyme and of the mesodermal lining of the coelomic cavities. These cavities, particularly the left one, are in turn destined to become major factors in the subsequent development of the larva and its metamorphosis into the adult animal. Thus, the anteriormost portion of the archenteron not only is an important topographical mark, but has also a very significant developmental function to perform.

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[1] *Hörstadius*, 1928; see footnote on page 249.

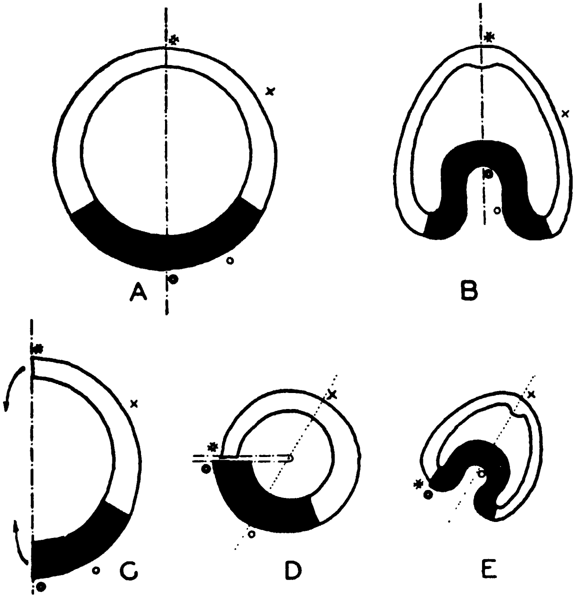

Fig. 50. Disposition of materials in the formation of a whole gastrula from a meridional half of a blastula in the sea urchin.

A, Whole blastula.
B, Whole gastrula.
C, Meridional hemisphere of blastula.
D, Hemisphere closed into sphere.
E, Gastrula developed from D.

Ectoderm white, entoderm black. —·—·— Original egg axis. .... New axis of germ. *, ◉, Original poles. ×, ○, New poles.

Now, compare this normal course of events with the development of a halved blastula (semi-blastula): The free edges of the bowl-shaped meridional half come to meet, fuse, and the germ regains the shape of a hollow sphere. Figure 50D shows the result. We see that the closure of the hemisphere into a sphere has brought into juxtaposition materials of the former animal (※) and vegetative (◉) poles. Thus the material of

> the prospective apical tuft and the prospective tip of the intestine which are normally antipodes have become next-door neighbors, as it were. This would cause serious confusion of subsequent development if these materials behaved in mosaic fashion and persisted in producing what they were originally destined to produce. What actually happens, is that *a normal and harmonious gastrula* forms (Fig. 50E) which has a gut of typical proportions on one side (○) and an apical tuft (×) on the opposite side. How the original materials of the semiblastula were utilized during this process can be seen at a glance by comparing the three stages illustrated in Figure 50C–E.
>
> It, thus, becomes obvious that although in general vegetative materials were used to build up the intestine, and animal materials to build up the ectoderm, there is no detailed correspondence whatsoever between the actual use to which individual portions have been put in the experimental germ and their prospective fate in a normal germ. The vegetative pole (◎), instead of becoming the front center of the intestine, remains somewhere near the outer rim (blastopore), while the actual apex with all its distinctive formative properties (see above) is composed of material (○) which under normal conditions would have formed some subordinate part of the wall of the intestine. Conversely, the material around the original animal pole (※) forms plain ectoderm and fails to produce a ciliary tuft, while the actual tuft develops in some material (×) which in normal development would have formed ordinary epidermis. If we project the new axis of the germ, which may be defined as the connecting line between apical tuft (○) and gut center (×), back upon the intact whole germ, we obtain a crooked line unrelated to the old axis. Conversely, the old axis (※–◎) drawn into the halved germ, appears bent back upon itself (Fig. 50D).

It would be easy to extrapolate from this description the fate of the other districts of the germ which we have neglected in this simplified picture; the result would show clearly that the fate of every single part of a bisected germ has been changed, as compared with a normal germ. It must be concluded, therefore, first, that the distribution of partial developmental tasks in a meridional half is entirely different depending on whether the half is isolated or whether it is in connection with the other half; and, second, that in either case the distribution is such as to lead to a larva of normal shape, normal proportions and normal equipment. *Larvae developing from separated meridional halves are not inferior to normal ones, except that they are of smaller size* (see p. 301).

"Regulative" development.

Eggs behaving in this fashion have been called *regulative* eggs. The term intimates that the eggs had started out toward the formation of a normal whole embryo, but that after experimental transection they were made over by regulative factors which could revoke the original assignments and deal out new ones more appropriate to the changed situation. An alternative view, however, is that the parts of the early egg contain no assignments at all, and that all details of development are left to be arranged at a later time. The difference between the two views is that, according to the former, the different parts of an egg are from the very start tentatively marked for certain uses, but the marks can be erased and replaced by others; while according to the latter, the parts of a young egg are completely "blank." We shall not enlarge upon this subtle distinction here; the main point to keep in mind is that neither of these views is reconcilable with a mosaic concept of development.

It is true that voices have been raised in favor of regarding regulative eggs as special cases of mosaic eggs. It has been suggested that every part of these eggs might contain, in addition to its regular commission designed for normal development, some kind of reserve commission which would not be activated unless in the contingency of a bisection of the egg, and that this spare mosaic in each half would be so pre-arranged that it could produce a whole embryo from the reduced material. With sufficient stretch of imagination one could conceive of several such reserve mosaics in the egg prepared to take care of a number of emergencies that might afflict eggs *in nature.* By no stretch of imagination, however, could one postulate that reserve mechanisms might have been preformed to meet contingencies of a kind that can never occur under natural conditions and which it was left to the experimentalist to contrive, as is illustrated in the following example.

Example 33.

A single larva from a double germ.

> If two whole sea urchin eggs, each of which would normally give rise to a complete embryo, are united in pre-gastrulation stages, *a single proportionate larva of giant size* can arise from the fused product. In order to obtain this result, it is necessary that the axes of the two partners coincide.[1] The double size of the resulting larva proves that the materials of both eggs have actually been utilized, and the harmonious organization proves that neither partner has contributed more than about half the number of organs which it would have normally produced.

Thus, the material of a single egg can be made not only into two fully developed larvae (example 32), but also into only one half of a larva. Since we cannot possibly assume that an egg is ever called upon under natural conditions to produce half a larva instead of a whole larva, the observations on fused eggs alone would invalidate any attempt to squeeze the regulative type of development into the rigid mosaic scheme. Increasing knowledge of the regulative types of eggs has in the meantime supplied us with even stronger arguments than this, but historically the first serious challenge to the mosaic concept came from the evidence of split and of fused regulation eggs.

Further "regulative" forms.

Development of twins from properly bisected germs has been observed in a wide variety of forms, among which are hydroids, nemerteans, some insects, fishes, birds, amphibians. For convenience, we shall call all these forms "*regulative*" types, as opposed to "*mosaic*" types, such as are represented by annelids, molluscs, ctenophores, ascidians. It will be noted that, in general, "regulative" type of development goes hand in hand with an indeterminate mode of cleavage, while mosaic development prevails in the groups with determinate cleavage (see p. 217). That this is not pure coincidence will be shown later (p. 412).

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[1] *Bierens de Haan*, 1913.

The implications of "regulation."

What do we learn from the "regulative" types of which the sea urchin may serve as the paradigm? Apparently, we learn two things: First, that in early stages *individual cells*, or even groups of cells, *are not yet earmarked for particular differentiations.* Lacking definite assignments, parts can be deflected from their normal destination and led into different channels of differentiation. Second, there must be certain factors operating in development which can *direct and coordinate the parts* in such a way that under a variety of conditions invariably the same typical product results, namely, an organism of the shape, organization, differentiation, and proportions which are typical of the species. It is important to keep in mind that neither of the two items alone could account for regulation; mere indetermination of an individual cell could not explain why eventually it does enter a definite course in conformity with the actual situation; and, on the other hand, even the most powerful directives would remain ineffective if the individual cells were not able to respond to them.

Harmonious-equipotential systems.

This *dual* aspect of regulation is expressed in the name given to the systems exhibiting it. They are called *harmonious-equipotential systems*, wherein "harmonious" refers to those factors which are responsible for the harmonious character of the ensuing development, while "equipotential" alludes to the equal readiness of all parts throughout the system to let themselves be properly directed. In a harmonious-equipotential system all parts are envisaged as of identical character and identical developmental capacity so that when they are arbitrarily interchanged, the shift will not affect the final result. This amounts to *postulating a practically homogeneous constitution* of the system in question. It is further implied that those harmonizing agents responsible for the *organization* of a normal developing system, and *pari passu* for the *reorganization* of an experimentally altered system, can force the originally equivalent parts into such a pattern, and impose upon them such behavior, as will *conform to the general plan of construction.*

It was primarily this formulation of the properties of harmonious-equipotential systems which tempted *Driesch,*[1] and the neo-vitalist school with him, to invoke a *non-material* principle as the controlling agent of development. This principle, called *Entelechy*, was assumed to have autonomous existence and to be merely in lawful counterplay with the material system of the organism. It seems that by accepting *Driesch's* philosophical dogma we would not only exceed our license as scientists but, at the same time, subscribe to an interpretation of the facts which they themselves do not warrant. It is true that we must apply a dual standard to the germ, depending on whether we focus on the individual elements or on their subordination to an integrated collective whole; but these are merely two different aspects of the same material system, germ. Nothing proves that only the elements have material physical existence, while the controlling agents are metaphysical and acting from without. On the contrary, we have discovered no facts that would conflict with the view that *the agents controlling development emanate from no other source but the corporeal system, "germ," and its counterplay with the material environment.* There is no reason, therefore, to assume that the organizing activities to which the individual parts of a germ react are manifestations of other principles than those residing within the confines of that same material system of which the reacting parts form the integral constituents. Even a harmonious-equipotential system must thus be regarded as carrying the conditions determining and controlling its development *within itself.* These remarks seem necessary to make it clear that the question of the existence of harmonious-equipotential systems can be cleanly divorced from their philosophical exploitation in favor of disembodied vital principles.

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[1] *Driesch,* 1929.

Testing harmonious equipotentiality.

Whether or not a given developmental system is harmonious-equipotential, is an empirical question. Pure observation, however, cannot settle the matter any more than looking at a metallic bead can disclose whether it is molten or solid. But by dividing the bead we can determine its state: if the fragments retain their shape, it has been solid; but if they round off, it has been liquid. Similarly, only the behavior of a germ after experimental interference can tell us whether it is more nearly in the plastic, self-regulatory, condition of a harmonious-equipotential system, or the rigid condition of a mosaic. Such experiments will now be described.

Dividing an egg into two *unequal* portions supplies us with a *defect* experiment plus an *isolation* experiment; the larger fragment is the one suffering the defect, and the smaller fragment the one being isolated (see p. 148). It may seem that a defect experiment is just as adequate a procedure to test the harmonious-equipotential character of an egg as is the splitting into equal portions. This conclusion is not quite valid, for the following reasons.

First, the infliction of a small defect to an egg causes less disturbance in the remaining portion than does the removal of a full half. A disturbance too weak to stir up the rest of the system may go unnoticed and fail to cause reorganization. This is often true even of large defects, as is illustrated in the following case.

Example 34.[1]

Apparent lack of regulation in the frog's egg.

When a hot needle is applied to one of the two blastomeres of a frog's egg of the two-cell stage, the heat coagulates the material contained in this blastomere and thus prevents it from developing further. The other blastomere, left in contact with the paralyzed one, can proceed with its development. At the end of gastrulation it is found to have produced *one half of an embryo* (Fig. 51); just what half depends apparently on how the first cleavage plane was oriented with regard to the future planes of symmetry of the embryo.

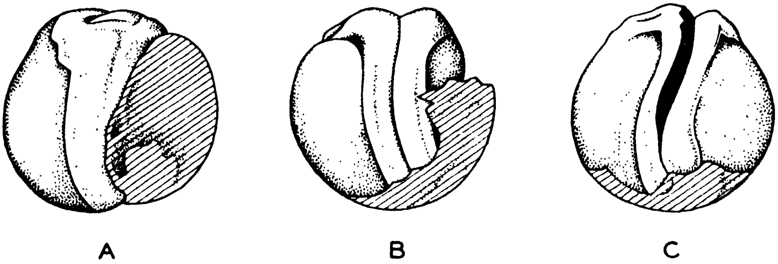

FIG. 51. Partial development of the frog egg after destruction of one of the first two blastomeres. (After *A. Brachet*) The embryonic parts suppressed by the elimination of one blastomere vary with the varying position of the first cleavage plane relative to the prospective median plane of the embryo (see Fig. 37).

The surviving blastomere has given rise: in A, to lateral half of embryo; in B, to oblique half; in C, to antero-ventral half.

In this experiment, the performance of the surviving blastomere was essentially what it would have been if the other blastomere had also developed. The fact that no regulation took place suggests a mosaic type of development. But it was soon realized that this lack of regulation was not to be ascribed to lack of regulative power or potency in the surviving blastomere, but rather to the fact that this blastomere had remained unaffected by the operation; hence, re-routing of the developmental processes so as to conform with the new situation has failed to occur. That this view is correct is demonstrated by the following example.

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[1] *Roux*, 1888; *Brachet*, 1905, 1917.

Example 35.[1]

The frog's egg can regulate.

> When frog eggs are turned upside down and fastened in this position so that they cannot right themselves, the heavier yolk (see p. 190), which is now uppermost, gradually sinks down into the lower hemisphere, while the lighter oöplasm rises to the top. If eggs are inverted in the two-cell stage, yolk streams down and oöplasm streams up, and not only does this agitate the substance of the two blastomeres thoroughly, but it also accentuates the partition between them, because some matter clings to the cleavage plane. Such eggs frequently produce *twin embryos*, that is, *one complete embryo from each half of the original egg.*

Since the difference between examples 34 and 35 consists primarily in that, in the former, the surviving blastomere of the two-cell stage was left undisturbed, whereas, in the latter, both blastomeres were thoroughly stirred, the *importance of a shake-up of the egg substance for the manifestation of regulatory capacity* becomes obvious.

Crucial tests of harmonious equipotentiality.

We shall return to this case on a later occasion (p. 347); it illustrates one reason why defect experiments are not always reliable as tests of the presence or absence of regulatory capacities. An isolation experiment, the complementary part of a defect experiment, is not always crucial, either, because the isolated parts may continue to develop in a very definite direction (i.e., manifest self-differentiating power), and yet possess at the same time a number of additional potencies which the isolation has not been an adequate method to bring out. Therefore, a *defect* experiment, and the complementary *isolation* experiment as well, can be suggestive, but never wholly conclusive, with regard to the presence of regulatory capacities and the latitude of potencies. It is to the *translocation* experiment and the *recom-*

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[1] *Schultze*, 1894; *G. Wetzel*, 1895; *Penners and Schleip*, 1928.

bination experiment (see p. 150), that we must look for crucial evidence as to whether or not a part has lost its responsivity to new demands.

ANALYSIS OF SEA URCHIN DEVELOPMENT

Different behavior of equatorial and meridional halves of an egg.

We have seen above that *meridional* halves of echinoderm eggs regulate into whole larvae (example 32), whereas *animal* and *vegetative* halves obtained by equatorial transection of the egg behave more like two members of a mosaic. In order to reconcile these results, one must assume (1) that the animal and the vegetative halves each possess properties which the other lacks; but that (2) *any portion* of these halves contains these properties. Since a meridional half contains both animal and vegetative materials in the normal proportions (Fig. 50), such halves can undergo a more complete development than can either animal halves or vegetative halves alone. It is obvious that, except for the segregation of some generalized vegetative and animal properties into the corresponding halves, there is no more detailed allocation of partial tasks within either half; otherwise, the extensive redistribution of tasks discussed above (p. 261) could not possibly have taken place. This conclusion is fully borne out by the following observations. What we aim to demonstrate is that *individual cells of the young germ do not yet contain irrevocable trends of differentiation.*

Example 36.[1]

Using animal cells for vegetative formations.

When a meridional half of a sea urchin egg is isolated in the sixteen-cell stage, it consists of four mesomeres, two macromeres, and two micromeres (Fig. 52), and contains animal and vegetative materials in the typical proportions, both being of half the normal size. We know from example 32 that such a meridional half would differentiate into a normal, proportionate, though undersized, larva. To this group of eight cells we add now the eight animal mesomeres of another germ (i.e., a full quota of animal material). Thus we obtain a composite germ of sixteen cells (twelve mesomeres, two macromeres, and two micromeres) in which the animal materials, representing prospective ectoderm, outnumber the vegetative materials in a ratio of 3 to 1. *Such germs develop into perfectly normal, proportionate, and well-differentiated larvae* with no evidence of the initial disproportion between vegetative and animal materials. Vital stains reveal that an equalization has been achieved through the *diversion of a large proportion of the excess animal material into the formation of vegetative derivatives.*

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[1] *Hörstadius*, 1928.

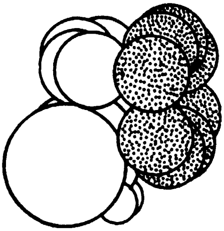

FIG. 52. Combination of a meridional half (white) with an animal half (dotted) of a 16-cell stage of the sea urchin. (See text.)

If the animal parts of these eggs had developed independently in accordance with their prospective fate, the resulting larvae would have had an ectoderm three times too large with regard to the vegetative derivatives. This has been avoided by the employment of some of the excess animal material in the formation of the gut and skeleton. This the animal cells would never have done in isolation. We thus arrive at the following conclusions.

First evidence of organizing activities.

The absence of vegetative organs in isolated animal halves reported in example 26 cannot be ascribed to an incapacity of the animal cells to join in the formation of vegetative structures. As we now see, *animal cells can participate in the formation of gut and skeleton.* Given this versatility of the animal material, we must still explain why only a definite proportion of it is transformed into entoderm and mesenchyme. In other words, it is not enough to know that animal cells *can* become intestinal or skeletal cells; there must have been something in the germ to direct them as to where, when, and to what extent to transform, so that they might fit into the pattern of the whole. The animal cells have multiple potencies, and, depending on the circumstances, one or another of these is activated. The "circumstances" include *specific organizing activities directing the individual cells according to a common structural plan.* It must be these latter factors which are either absent or suppressed in isolated animal halves; otherwise there would be no reason why such isolated halves should fail to form normal germs, despite the demonstrated ability of their cells to differentiate vegetative as well as animal structures.

Using vegetative cells for animal formations.

Whereas in example 36 the cells of the animal hemisphere were forced to enter into vegetative differentiations, the reciprocal phenomenon has also been produced, namely, the *formation of typically animal derivatives by vegetative cells.*

Example 37.[1]

> In a sea urchin egg of the sixteen-cell stage the vegetative half, consisting of four macromeres and four micromeres, is isolated, as in example 26; then the micromeres are cut off, leaving only the macromeres for further development. They continue to divide; a blastula is formed; gastrulation occurs, and finally a larva appears which usually is fully equipped; that is, in addition to the usual *vegetative* derivatives — gut and skeleton — it possesses *a ciliary tuft, ciliary bands, and stomodaeum,* all of which are normally produced by cells of the *animal* hemisphere.

This result shows that at least *some of the cells derived from the vegetative half have the capacity to assume typical animal characters.* It should be mentioned that this transformation becomes the more difficult the closer to the vegetative pole the cells are situated; in other words, there is a gradient in the readiness of cells to let themselves be directed away from their original courses of development. Fundamentally, however, cells throughout the germ seem to be similar in that they are amenable to a variety of differentiating influences, albeit with different degrees of facility.

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[1] *Hörstadius*, 1935.

Again one may ask why the cells of a vegetative half, although capable of producing animal organs, fail to do so when the whole vegetative half is isolated (example 26). A comparison between examples 37 and 26 reveals a seeming paradox: a vegetative half produces a much more normal larva when deprived of the micromeres (example 37), than when left in possession of them (example 26). One cannot say, therefore, that a vegetative half lacks *per se* the organizing capacity necessary to make a normal individual. Rather we must conclude that the presence of micromeres somehow interferes with the tendency of the isolated vegetative half to form a whole and harmonious larva. This conclusion has been corroborated by a number of experiments.

The organizing effect of vegetative fragments.

Fragments of the vegetative hemisphere, therefore, contain all the factors necessary to organize the development of a whole individual. Our interest is thus turned from the more or less uniform reactivity of the cells, to those specific factors which cause each cell to follow just one definite course of the many open to it. Where are these factors located, and what do we know about their operation? For brevity, let us call them *organizing factors*. Pending a later qualification, this term will be used to refer to all actions through which the developmental course of the parts of a germ is fixed so that it complies with a certain orderly pattern of development.

Such actions have been recorded in examples 31, 32, 33, 36, and 37. The only portion of the germ present in all of these cases is that part derived from the *macromeres*, and, as can be seen from experiments 32 and 36, even half of this portion is effective. It would seem logical, therefore, to attribute the organizing effect to the macromeres exclusively. The following example, however, shows that there is no such monopoly.

Example 38.[1]

Micromeres reorganize an animal half.

We know that an isolated animal half of a sea urchin egg can form only a ciliated sphere of ectoderm (example 26). We also know that the micromeres when isolated in the sixteen-cell stage merely divide a few times and form a cluster of cells with no recognizable differentiations. If, however, these micromeres are transplanted into an isolated animal half, *practically normal individuals can develop.* Detailed studies have revealed that the micromeres themselves form only the skeleton of the larva, while the gut is differentiated from the animal material near the site where the micromeres have been incorporated.

Reviewing this case, we note: An animal half alone forms no larva; micromeres alone form no larva. But in combination they produce a larva. In striking contrast to mosaic behavior, the product of their combination is by no means a mere sum of what they would have produced in separation. The material contribution of the micromeres was confined to skeleton. But much more than skeleton would have been lacking in the isolated animal half; there would have been no gut, no typical shape and arrangement of the animal parts. All of this has now been produced, and among animal cells. Hence, the micromeres, in addition to contributing a modest share to the building material, have taken the lead, as it were, and made the animal cells supplement the missing vegetative organs. Specific influences of this sort, by means of which *one tissue calls forth specific formations in an adjoining tissue,* are customarily referred to as "*inductions*" (see p. 335). So, we can say that the transplanted micromeres have *induced* the formation of vegetative organs in the surrounding animal half. The fact that the micromeres have at the same time furnished the material for the skeleton is irrelevant because, as we have seen in example 31, other materials could ably substitute here for the micromeric material.

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[1] *Hörstadius*, 1935.

The effect of the implanted micromeres is not confined to their immediate vicinity where it causes the gut to appear, but apparently embraces the whole protoplasmic system. Thus the apical tuft, which in isolated animal halves expands practically over the whole surface (Fig. 45), is after the addition of micromeres reduced to approximately its normal extent. The micromeres, therefore, restrain the development of the animal organs in addition to stimulating the formation of vegetative organs.

Do micromeres act "intelligently"?

To a superficial observer the action of the micromeres, which converts freakish animal hemispheres into fairly normal individuals may easily look like a deliberate act, and the term "regulation" given to phenomena of this sort is rather suggestive of this view. Drawing a parallel to human actions, one might think that the micromeres, endowed with special powers and faculties, enter the confused system, take stock of the situation, and then begin to deal out order and specific instructions in accordance with what they have decided to be the best way out of the predicament. Some very competent and serious biologists of the past have actually considered regulation under this anthropomorphic viewpoint. However, it must be stated quite explicitly that such views are based on grave misconceptions. The micromeres, of course, have no mind; so, they cannot have the end of development in mind and what would best serve it. They do not possess insight into the situation and cannot devise remedies to meet each emergency by appropriate measures. *They act blindly and with no regard to the ultimate usefulness of their results to the individual specimen.* This is best demonstrated by the fact that they can be tricked into very inadequate performances by being made to act in wrong places, where they continue to reproduce the one performance of which they are capable and which, being devised for normal circumstances, is out of place. In other words, regulation, in the sense of intelligent capacity to cope with any contingency, does not exist. As one example from many we may cite the following.

Example 39.[1]

Extra micromeres spoil the harmony of development.

> An isolated set of micromeres of a sea urchin egg is implanted into a normal full-sized germ possessing its own micromeres, instead of into an isolated animal half, as was done in the previous example. If the graft is incorporated some distance from the vegetative pole of the host germ, it *establishes a second center of invagination;* a second intestinal tract appears, as well as supernumerary skeletal elements.

In other words, the addition of this numerically inferior cell group has caused considerable confusion and disturbance in the normal germ. It started to organize another set of vegetative organs, notwithstanding the fact that already one such set was being elaborated in the normal location, apparently in response to similar actions emanating from the normal vegetative pole. Thus, the organizing effect of the micromeres, which appeared so highly profitable to the germ when exercised in an isolated animal half, can be distinctly detrimental in other circumstances; viewed objectively, the action is, however, the same in both cases.

A gradient of vegetative organizing power.

It is obvious from these last examples that the micromeric material is capable of exerting a very positive, specific effect upon its surroundings, leading to the organization of typical vegetative organs. On the other hand, (example 31) vegetative organs also form in the absence of micromeres. Hence, we must conclude that neither macromere nor micromere material has a monopoly on this organizing effect. Ap-

[1] *Hörstadius*, 1935.

parently *one cannot point to any particular cell as the residence of the organizing influence.* Rather we are dealing with a more or less *diffuse property;* further information concerning this property is obtained from the following example.

Example 40.[1]

> Although all parts of a vegetative half are endowed with organizing capacity, all are not equally potent. In the stage following the thirty-two cell stage the vegetative half consists of two rows of divided macromeres (mac_1 and mac_2) and the micromeres (see p. 246 and Fig. 43). This provides us with three different levels of vegetative cells, whose relative efficiency in organizing a germ can be tested by combining each one separately with an isolated animal half; the organization subsequently attained by the latter can serve as a measure of the organizing power of the implanted vegetative fragments. We already know the effect of the micromeres: complete organization (example 38). Testing the lower row of macromeres (mac_2), one finds them fairly potent in organizing an animal half; but the induced vegetative organs show a certain weakness and reduction in size, which proves that the material in mac_2 is not quite as powerful as the micromeres, especially if we consider its larger mass. By combining an animal half with the upper row of macromeres (mac_1), larvae are obtained in which the presence of vegetative organs is barely indicated; the intestine, if occurring at all, remains abortive, and the skeleton, if present, remains inconspicuous. On the other hand, the animal organs — apical tuft, stomodaeum, and ciliary bands — are of normal proportions. Thus, this most equatorial vegetative material (mac_1), although not very potent in producing vegetative organs, can, at least, prevent the disorganization which otherwise characterizes isolated animal halves.

These results reveal a *steady decrease of the organizing power with increasing distance from the vegetative pole.* The closer to the pole the fragments are chosen, the greater is their organizing effect. The fact that the intensity of the organizing effects depends on the *distance from a certain center* reveals that we are not dealing with activities of an "all-or-none" type — an important clue in the search for the organizing factor.

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[1] *Hörstadius,* 1935.

Further examination shows that the effect is in a certain direct relation to the *mass of material* exerting it. Thus, two micromeres are more effective than one, and four micromeres are more effective than two. One micromere, added to an animal half, restrains the animal formations to more nearly their normal bounds, but no gut or skeleton appears. Two micromeres are strong enough to produce some semblance of a vegetative differentiation. Four micromeres, as we have seen in example 38, are all that are needed to make a proportionate larva (Fig. 53).

A gradient of cellular responsiveness.

But the results are conditional not only on the organizing power of the micromeres, but also on the readiness of the reacting tissue to differentiate entodermal structures; and in this regard there are again inequalities among different levels of the germ. The ease with which, for instance, gut can be induced, decreases as we go from the vegetative to the animal pole, so that it requires a stronger action to organize fragments from more animal levels than is necessary for the more vegetative ones. While it takes four micromeres to provoke a proportionate entoderm in the most animal portion of a germ (example 38), two micromeres are sufficient to produce the same result in the isolated lower (near-equatorial) ring of mesomeres (mes_2; see Fig. 43), and even one micromere, though not strong enough to produce a perfectly normal result, organizes a little gut and skeleton. Implanted into the isolated upper ring of vegetative macromeres (mac_1; Fig. 43), even a single micromere is adequate to organize a larva which, save for the imperfection of some animal parts, is well formed. These facts are summarized in the diagram, Figure 53.

Activities of the animal half.

The fact that the number of micromeres necessary to bring about a given result varies, depending on the material with

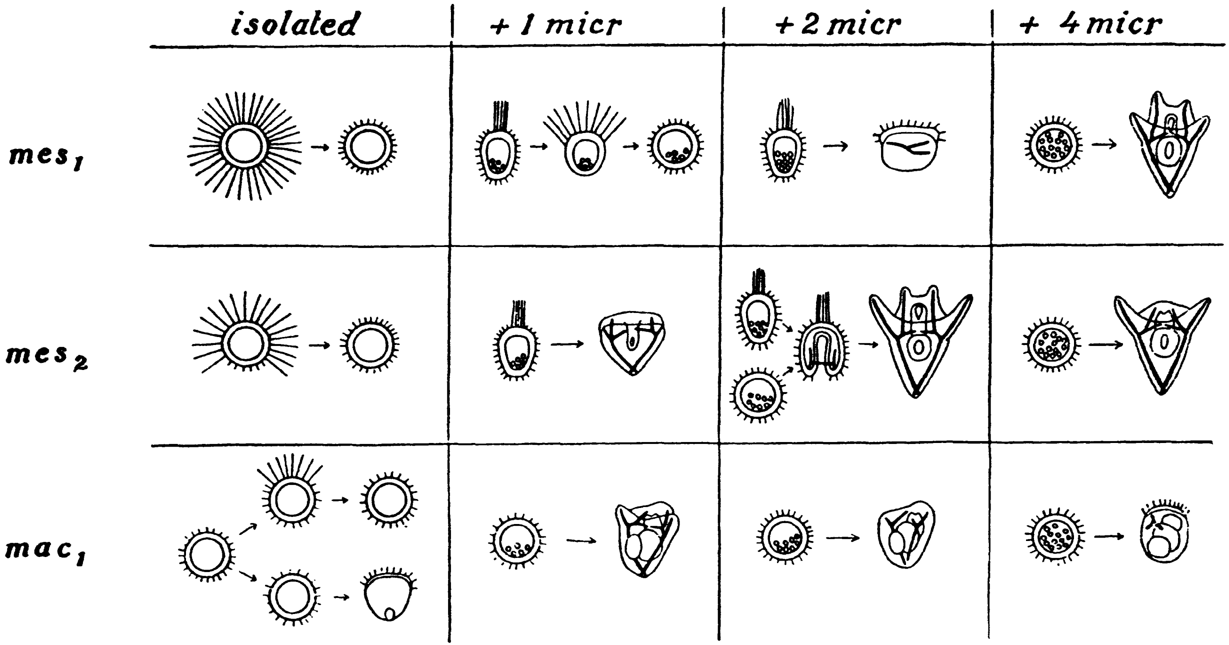

FIG. 53. Fate of different levels of the sea urchin germ isolated (first column) and in combination with micromeres (second to fourth columns). (From *Hörstadius*, 1935) Abbreviations as in Fig. 43.

which they are confronted, indicates that they are working against varying resistance. This resistance which increases in the direction of the animal pole is the manifestation of an *antagonistic force or factor which is engaged in the organization of animal organs and centers around the animal pole.* The effectiveness of this action decreases with increasing distance from the animal pole. This becomes evident from Figure 53. The further away from the animal pole the isolated materials were chosen, the less inherent animal tendency had apparently to be overcome before cells could be diverted into vegetative formations, and hence the fewer micromeres were required to produce this effect.

This fact introduces another complication into the picture. Now we realize not only that no particular part of the vegetative half has a monopoly of organizing power, but that organizing capacities as such are by no means confined to the vegetative half. The animal half possesses a similar set of factors; even though they are less conspicuous and probably less potent than those emanating from the vegetative half, they undeniably are there. The unchecked expansion of these animal factors explains why in an isolated animal half all cells tend to develop large bristles, a character normally restricted to the cells at the animal pole (p. 249, Fig. 45). The animal factors simply overwhelm the isolated animal half unless they are held within bounds by antagonistic actions emanating from added vegetative material (p. 274). These animal factors are more directly demonstrated by the following experiment:

Example 41.[1]

If an isolated animal half of a sea urchin egg of the 32-cell stage is combined with two of the eight *macromeres* (Fig. 54A), the resulting larva shows some trace of *vegetative differentiation.* If only *one macromere* is used instead (Fig. 54B), *no invagination* occurs, and development of the animal parts is frequently

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[1] *Hörstadius,* 1935.

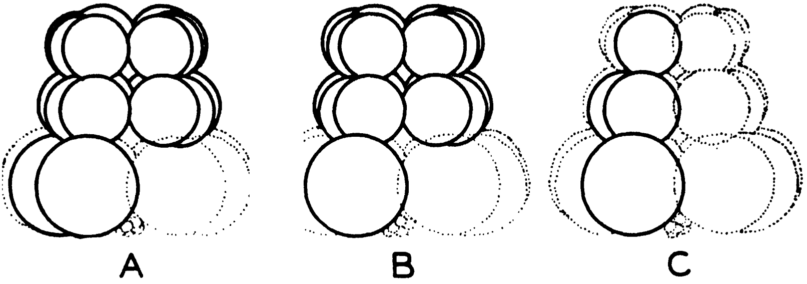

FIG. 54. Removal of blastomeres in the stage following the 32-cell stage of the sea urchin. (After *Hörstadius*)
A, Removal of mic and $\frac{3}{4}$ of mac.
B, Removal of mic and $\frac{7}{8}$ of mac.
C, Removal of mic, $\frac{7}{8}$ of mac, $\frac{7}{8}$ of mes_1, and $\frac{3}{4}$ of mes_2.
Abbreviations as in Fig. 43.

exaggerated. If, however, this same amount of vegetative material — *one macromere* — is confronted with only *three-sixteenths of the animal half* instead of with the entire animal half (Fig. 54C), *invagination occurs, and a fairly normal larva can develop.*

One is thus forced to conclude that one macromere contains sufficient vegetative power to produce gut and skeleton, but cannot assert itself against the overpowering activity of an entire animal half. If, however, the latter activity is weakened by reducing the animal tissues to less than one-fourth their normal mass, balance is re-established, and harmonious development can ensue.

The competition between animal and vegetative factors.

As a result of all these experiments the opinion has crystallized that the primordial organization of the sea urchin egg occurs under the influence of *two antagonistic actions, one centering around the vegetative pole and directing the development of vegetative parts, the other centering around the animal pole and directing the animal differentiations.*

We can compare the two opposing sets of factors to two opposing armies contending for the possession of limited land. The land is the cellular material of the germ. For this material the two armies compete. The actual boundary between their spheres of influence will be determined by their mutual strength. Strengthening one amounts to the same as weakening the other, and the boundary will immediately shift in the direction of the weaker center. Thus the boundary is no steady mark such as separates peaceable neighbors, but is a fluctuating line along which the fighting parties have come to a stalemate. Consequently, the position of this front line permits an estimate of the relative strength of the two partners. Inasmuch as the strength both of the animal and the vegetative factors decreases with increasing distance from the corresponding poles (see pp. 276 and 279), there must be a line somewhere part way between the poles along which the forces are in equilibrium. To the animal side of this line the animal factors prevail, and to the vegetative side of it the vegetative factors overpower the animal ones. On either side, the dominating factor claims the material for its own specific use.

Shifting the vegetative-animal balance.

Dwelling on this analogy, we recognize that, just as the striking power of an army can be reduced by reducing either the numbers or the efficiency of its individual units, so the effectiveness of the animal and vegetative factors of organization can be scaled down by reducing either the *mass* of active materials or the *intensity* of their activities. In examples 37 to 41 numerous instances have been mentioned in which the normal proportions between vegetative and animal organs had been thrown out of balance by the disproportionate reduction of the *mass* of either the vegetative or the animal system in the egg. This covers the first point. As for the second point, it has been shown, in fact, that the *intensity* of the animal or the vegetative processes can also be lowered without diminishing the substance of the egg at all. This has been achieved by the use of certain chemicals.

Example 42.

A chemical that favors the vegetative powers.

If sea urchin eggs are immersed for some time in a solution of lithium chloride and then are returned to normal sea water, they generally develop into abnormal larvae.[1] In extreme cases the entoderm fails to invaginate (Fig. 55A), and the archenteron is turned inside out (exogastrulae; see example 19). This has been shown to be due to the *excessive size of the entoderm as compared with the ectoderm;* it would be mechanically impossible to invaginate two-thirds of the surface of a sphere into the remaining one-third. A weaker dosage of the treatment

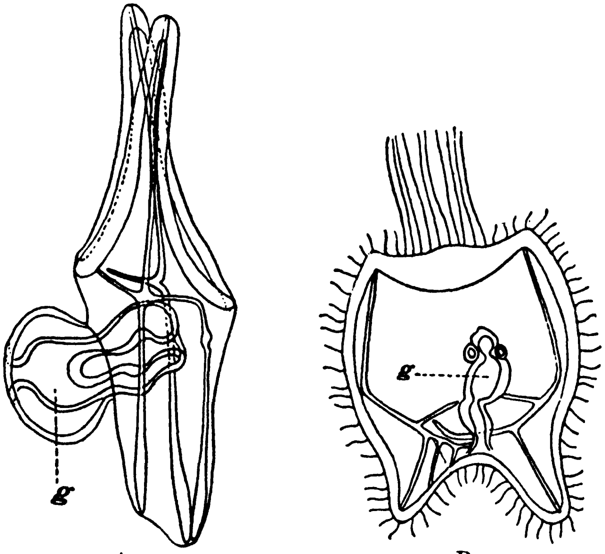

FIG. 55. Chemical dislocation of the balance between animal and vegetative formations in the sea urchin, *Paracentrotus lividus*. (From *Lindahl*, 1936)

A, Animal depression by Lithium: Egg transferred after fertilization into weak solution of LiCl in sea water and returned to normal sea water at the end of 24 hours. Excessive development of vegetative organs with oversized, partly evaginated gut (*g*). × 150.

B, Vegetative depression by NaSCN: Egg placed in weak NaSCN solution in sea water for 15 hours and then fertilized produces oversized animal parts; gut (*g*) abortive. × 280.

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[1] *Herbst*, 1893; *MacArthur*, 1924; *Lindahl*, 1936.

produces larvae with *disproportionately large vegetative portions,* but still some semblance of normal form.

The less severe cases of this set resemble larvae obtained from isolated vegetative halves (cf. Fig. 46A), and this resemblance suggests similar causation. That is to say, *the action of lithium and the cutting away of animal material have approximately the same effects.* In both procedures the vegetative forces have been given an advantage over the animal tendencies, and, as a result, the border line between entoderm and ectoderm has been shifted into the animal hemisphere. It is noteworthy that this shift not only can be deduced from the later disproportions of entoderm and ectoderm in the larva, but is already discernible in the egg: as described in example 19, dark field observation of Li-treated eggs reveals an actual expansion of the vegetative surface character far into the animal hemisphere.[1] Thus an unbalancing of the prospective ectoderm and entoderm districts in the egg anticipates the later disproportion among their derivatives. It is impossible to tell from these experiments whether the additional margin given the vegetative over the animal factors comes from a strengthening of the former or a weakening of the latter; but there are indications that the primary effect of the treatment is a *depression of the animal activities,* which secondarily enables the expansive vegetative tendencies to claim more land than they would have ordinarily succeeded in controlling. The most impressive demonstration of the differential depression of animal activities by lithium ions comes from the reaction of isolated animal halves to such treatment.

Example 43.[2]

Restoring vegetative-animal equilibrium to isolated animal halves.

Isolated animal halves which, as shown in example 26, would differentiate no vegetative organs, were exposed during predifferentiation stages to appropriate concentrations of lithium salts. This had the remarkable effect of producing a more or less extensive *invagination, followed by the formation of gut and skeleton* (Fig. 56).

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[1] *Runnström,* 1928a.

[2] *v. Ubisch,* 1929.

Thus, one is confronted with the seemingly paradoxical situation that an agent — lithium ions — which causes decidedly abnormal development when applied to a whole germ, leads isolated animal halves to a much more nearly normal course of development than they would have otherwise followed. The solution is quite simple, however. Animal halves suffer from the prepotence of the animal forces. Lithium action, by weakening them, restores a more normal balance and thus gives the feeble vegetative tendencies a chance to assert themselves. The effect obtained here through the *weakening* of the *animal* activities resembles that obtained by relative *strengthening* of the *vegetative* activities following the implantation of micromeres into animal halves (example 38).

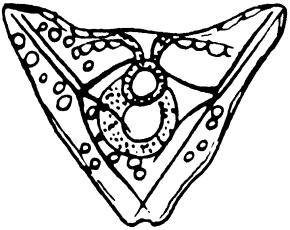

FIG. 56. Effect of Lithium treatment on isolated animal halves of the egg of the sea urchin, *Echinocyamus*. (From *v. Ubisch*, 1929) The upper four blastomeres of an 8-cell stage (see Fig. 43c) were isolated and reared in a weak solution of LiCl in sea water. Instead of the usual fragmentary development illustrated in Figure 45, more normal larvae with gut and skeleton were obtained.

A chemical that favors the animal powers.

While lithium ions, at least in pre-gastrulation stages, depress the animal processes, other chemical ingredients have a depressing effect upon the vegetative processes.[1]

Example 44.[2]

Sea urchin eggs exposed prior to fertilization to solutions of sodium sulfo-cyanide (NaSCN) and then reared in ordinary sea water are notable for the *weakness of their vegetative development while the development of the animal parts is excessive.* In this respect the effect is the reverse of that obtained by lithium treatment. Lack of calcium ions in the medium predisposes the germ for the effect. If eggs that have been treated with NaSCN are later additionally exposed to lithium ions they develop into *larvae of more normal construction* than those obtained after either sulfo-cyanide or lithium treatment alone. Evidently, *the effects of both agents cancel each other,* comparable to the proportional reduction of animal and vegetative materials resulting from meridional splitting of an egg.

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[1] *Runnström*, 1925a.
[2] *Lindahl*, 1936.

These results make it clear that the effects of the various chemicals in question cannot be explained simply on the assumption of an indiscriminate weakness or susceptibility of some parts of the germ toward toxic agents in general (see p. 159). For the chemicals here employed act on *different* parts of the germ. Lithium ions affect the animal half, while sulfo-cyanide ions weaken the vegetative actions. From this it has been inferred that these ions interfere with the chemical substrates of the animal or vegetative processes, respectively, and that their action, therefore, is one of specific rather than of general toxicity. It is obvious that if both were merely toxic in a general way, their joint administration should have additive rather than neutralizing effects. But, in reality, one is an antidote against the other, so far as the developmental results are concerned.

Terminology.

Let us, now, recapitulate the fundamental points brought to light by the preceding discussion. Here is a dictionary of the terms which will be used in this summary for convenience:

Germ — the egg throughout its development and with no regard to the actual developmental stage.

Early germ — the germ up to the blastula stage.

Late germ — a germ in which gastrulation has set in.

Parts — any arbitrary subdivisions of the whole germ.

This may denote an area covering a considerable fraction of the surface, or small groups of cells, or individual cells. Thus, there is a hierarchy of parts, the smaller ones being contained in the larger ones, while again the latter form part of a still larger unit.

Prospective, or *presumptive, fate* — the destination towards which we know from previous experience that a given part would have developed under ordinary, i.e., undisturbed, conditions (see p. 244).

Differentiation potency — the total repertoire of cytological and histological differentiations at the disposal of a given cell (see p. 100).

Unipotent — the attribute of a cell which can only give rise to one *single* type of differentiation. This type, of course, is identical with the presumptive fate.

Pluripotent — the condition of a cell which is amenable to *several* courses of differentiation.

Omnipotent — the attribute of a cell which can assume *every* histological character known to the species, or which by division can give rise to such cells.

Summary: Progressive organization of the sea urchin egg.

In terms of this language, the results of the above studies can be formulated as follows:

1. In the early germ the *differentiation potency* of any given cell is not limited to the one course indicated by the prospective fate of that cell. At this early stage cells are decidedly *not unipotent.* They are *pluripotent,* and some are even *omnipotent.*

2. Accordingly, numbers of cells at this stage are *equipotent* as concerns cytological differentiation.

3. The same statements as made for the individual cells hold true for *cell groups.* Some areas in the early germ are as *devoid of definitive morphogenetic tendencies* as the individual cells are lacking definiteness with regard to cytological differentiation.

4. Consequently, the cellular materials of the early germ

could produce a practically *infinite variety of morphogenetic* and *histogenetic* results.

However:

5. They *do* produce only *one definite pattern* from among the many. This pattern is the standard pattern of the species. It implies *definite localization, correct timing, and proper intensity* of the morphogenetic and histogenetic processes. That only this restricted selection of potencies is put into effect, is due to the operation of *organizing factors* in the germ.

6. Certain *large fragments of the early germ* — for instance, meridional halves or quarters — contain, after isolation from the rest of the germ, a *complete set of those organizing conditions which determine the typical pattern of a total animal.* This proves that in the early germ the factors underlying the total pattern of organization are not yet subdivided into local groups of different tendencies, as later in the mosaic stage they will be. Each major fragment of an early germ, when isolated, may carry with it a full array of organizing factors of evidently the same combination, configuration and balance as characterize a whole germ; the presence of these factors in full integrity permits the reduced system to develop into a smaller, but otherwise proportionate and typically organized individual.

This is a pure statement of fact. A magnet, broken in two, gives a vague picture of the situation, but evidently in immensely simplified conditions. Each of the two fragments of a magnetized rod becomes itself a magnet, equivalent to the original magnet in activity and polarity. The inert middle of the original rod emerges from the break as the south pole of one and as the north pole of the other fragment. Inactive matter has thus become the seat of powerful action by a mere break in the continuity of the system. This provides a general picture of how the properties of the parts of a system depend on their location within the whole; but that is about all we can gain from the analogy, because neither are the organizing forces in an organism of magnetic nature nor is the pattern of their distribution and activities as simple as the mere axial differentiation of the magnet.

7. Our conclusion, then, is that the forces and factors organizing an isolated half egg into a whole larva are the same as the ones that under normal conditions organize the whole egg into a whole larva. Regulation in this view appears simply as a *manifestation of the regular developmental factors* rather than as a separate faculty.

8. Under the influence of the organizing factors, the *individual parts of the germ gradually acquire typical local assignments.* The earliest subdivision apparently consists of the segregation of an animal and a vegetative sphere of influence. All experiments have indicated that the outline between these two domains is neither sharp nor fixed, but is maintained dynamically as the line along which two antagonistic influences are in equilibrium. Further subdivisions within these areas appear later; for instance, the localization of the apical tuft, of the stomodaeum, and of the ciliary bands within the animal district; the outlining of the archenteron against the surrounding ectoderm in the vegetative district; the subdivision of the primitive gut into its three portions, the production of the coelomic pouches from the top-most portion of the archenteron, and the local condensation of skeleton-forming material; and concurrently, the establishment of a definite dorso-ventral disposition of these features.

9. Once these subdivisions have actually appeared, the germ has lost the character of an harmonious-equipotential system. Whether this is due to the loss of equipotentiality on the part of the constituent cells, or rather to the dissipation of the "harmonizing" factors, is a question which will be discussed later on a more suitable material. The effect is the *gradual emergence of a mosaic condition* enabling individual fragments of the *late* germ to proceed by self-differentiation. The parts of the late germ have become single-tracked and pursue each one a definite job which has been allocated to them during the earlier phase of development.

GRADUAL DETERMINATION AND THE FIELD CONCEPT

Developmental Dynamics

The epigenetic determination of cell fate.

The most important outcome of these studies is the realization that *individual cells owe their fate not to their lineage*, that is, to their being derived from a particular parcel of the undivided egg, *but rather to the operation of forces and conditions to which they become subject in the course of development.* Some clues concerning the operation of these organizing factors can be gathered from the experiments. So, one notes that in the earliest stages accessible to experimentation there are already two distinct groups of such factors present, an animal and a vegetative group, associated somehow with the animal and vegetative materials. They center each on the corresponding pole, which immediately raises the question as to whether the "poles" might not be the *residences* of agents generating organizing effects. This can be definitely denied, most crucially on the evidence of meridional transections of eggs (p. 261, cf. Fig. 50).

The dynamic nature of organization.

One remembers that in meridionally transected eggs the original animal (⨳) and vegetative (◎) poles were forced into close apposition with no telling effect on the organization of the larva. A new animal center (×), marked by the apical tuft, and a new vegetative center (o), marked by the focus of invagination, established themselves at some distance from the old poles, while the latter lost all prominence. The new centers appeared in what after the operation had come to be the foci of the reduced animal and vegetative areas. In other words, *the actual extent of an active district determines the position of its center.* Accordingly, it would be vain to search for any fixed material point in the egg as the "seat" of organizing action.

This is highly significant. For, it directs our attention from a geometrical to a *dynamic* concept. In this, the animal pole appears, instead of as a prominent particle from which special actions emanate in all directions, as an imaginary center in which we visualize as concentrated the organizing activities which in reality span the whole animal district. The point itself has no more material reality and distinctiveness than have the center of gravity of a physical body or the population center of a country. Just as every change in the shape of a physical body shifts its center of gravity, so the imaginary vegetative center, which in a normal egg coincides with the vegetative pole, appears in a new position after the vegetative district has been halved. *Any shift in the outlines and proportions of those material substrata with which the animal and vegetative activities are connected must cause a displacement of their focal points.*

The field concept.

Now, after having gained some familiarity with these "*organizing factors,*" it seems timely to find a suitable name for them, both for convenient reference and in order to emphasize the reality of their existence. Briefly, once more, they are the factors which *cause the originally indefinite course of the individual parts of a germ to become definite and specific,* and, furthermore, cause this to occur *in compliance with a typical pattern.* Such factors have been called "*fields.*"[1] Thus we speak of the "animal field" and the "vegetative field" of the echinoderm egg, when we wish to refer to the activities organizing the animal and the vegetative organ systems.

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[1] In his first description of the "organizer" effect, *Spemann* (1921, on p. 568) uttered the remark that it appeared as if "a field of organization" is established by the "organizer." Although he neither qualified the term nor amplified its meaning, Spemann was undoubtedly the first to use it in connection with organization. Independently and almost simultaneously, *Gurwitsch* (1922) advanced a somewhat related idea. Coming from a third direction, the study of regeneration, *P. Weiss* (1923) established the field character of organization and elaborated the concept to include ontogeny (1926, 1935b). The field concept has been extensively adopted by embryologists.

Fundamental field characters.

A *field* is the condition to which a living system owes its typical organization and its *specific* activities. These activities are specific (p. 149) in that they determine the *character* of the formations to which they give rise. In this they differ essentially from what we have called trigger reactions. Since it is inconceivable that order could emerge from an irregular, chaotic play of forces, and inasmuch as the action of fields does produce spatial order, it becomes a postulate that the *field factors* themselves possess definite order. The *three-dimensional heterogeneity of developing systems,* that is, the fact that these systems have different properties in the three dimensions of space, must be referred to a *three-dimensional organization and heteropolarity of the originating fields.* In this respect the organization of a field bears some remote resemblance to that of a crystal whose electrical and optical properties also differ distinctly in different directions.

From the behavior of fields, such as the animal and the vegetative field of the sea urchin, it can be seen that each field has a focal point in which its intensity reaches a maximum; with increasing distance from this center the field intensity declines. This gradual decrease of the field intensity around the imaginary field center has led to the conception of field *gradients.* The field gradient in any given point of the field can be defined as that direction along which the field intensity falls off most rapidly. It must be borne in mind, however, that field gradients are merely convenient *symbols* to indicate the direction and rapidity of the decline of the resultant field action; as physical entities, they are just as fictitious and non-existent as is the field center (see above).

An appraisal of the field concept.

Before continuing with the description of further properties of fields, it seems advisable to put the meaning of the field concept in the correct light lest the use of the term arouse unwarranted illusions and expectations. The *field concept* is an abstraction trying to give expression to a group of phenomena observed in living systems. Essentially it is but an *abbreviated formulation of what we have observed.* Being an abstraction, we cannot expect it to return more than what we have put into it. Its *analytical* and *explanatory* value, therefore, is *nil.* Its *utilitarian* value, however, is considerable. It permits us to bring a certain, if only temporary, order into the observed facts; moreover, the consistency of the order thus obtained convinces us that the underlying principle cannot, after all, be mere fiction but must have real existence.

The field concept has its roots in purely *empirical* ground; i.e., the properties of fields are reconstructed from the observed facts; in this manner, such field attributes as *individuality, heteropolarity, gradation,* have been arrived at. Only secondarily, in a move toward hypothesis, has the attempt been made to inject *physical* sense into the *symbolic* term. The fact that practically all developmental phenomena exhibit field-like characters in one or the other respect, is, indeed, a strong indication that the field concept is not only a useful circumlocution, but an expression of *physical reality.*

Once we have gained this conviction, we raise the term to the dignity of an *object of research.* That is, if we acknowledge the real existence of such a thing as a field, this immediately imposes upon us the obligation to study it under all angles, just as we would study any other newly discovered phenomenon of nature. By outlining their occurrence, properties, limitations, and transformations, one tries to bring them within reach of rational scientific approach. If the term field were mistaken for a sort of narcotic devised to appease the mental discomfort arising from our profound ignorance of the problem of organization, its use would be highly inexpedient. If, however, one avoids this mistake, refrains from misleading claims, and considers the field merely as an *object for further research,* as something which apparently exists and whose properties and characters can be determined and described, then the term deserves legitimate scientific standing. Only in this strict sense do we propose to use it in the following.

The properties of fields.

We turn now to an outline of the principal properties of fields:

1. *Field activity is invariably bound to a material substratum.* We cannot accept the view advanced by some authors[1] that fields have an existence of their own independent of the materials upon which they act, because it does not seem that this latter, strictly vitalist, notion can draw any conclusive support from the observed facts (cf. p. 265). Consequently, a field exists only so long as the material substratum exists with which it is connected, and it dies with the latter.

2. *A field is primarily an entity and not a mosaic.* Its structure has the capacity of *self-conservation,* and in this regard it is merely a special kind of what we have called above (p. 111) physical *systems.* The stable structure of a field, i.e., its equilibrium, is characterized by a definite and unique distribution of properties, the *field pattern.* Following external disturbances this pattern can be restored, within limits, to its typical original form.

3. Fields, at least in the most specialized forms, are *heteroaxial,* which means that their structure varies along the three coördinates of space, and *heteropolar,* which means that their effects differ in the two opposite senses along the same axis.

4. A district whose activities show field character may be called a *field district.* A field district is characterized by the fact that none of its elements can be identified with any particular component of the field, although the *field as a whole* is a definite property of the *district as a whole.* Just remember the oversimplified but instructive example of the

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[1] E.g., *Gurwitsch.*

magnet in which it is likewise impossible to identify the dynamic characters, "south pole" or "north pole," with any intrinsic differences of the iron particles at either end.

5. When the mass of a field district is reduced, this does not affect the structure of the field as a whole. While the absolute limits of the field shrink, *its proportions remain unaltered* (e.g., example 31).

6. As a corollary of (5), the splitting of a field district into two halves leaves each half in possession of a complete proportionate field equivalent in structure to the original single field (e.g., example 32).

7. Fusion of two field districts into one can yield two types of results. If the two districts are brought together in such orientations that their axes coincide, and if both carry fields of identical character, the two fields will merge into one common field of the same structure as that owned by either of the contributors (e.g., example 33). If, however, the fields are of different character or differ in their axis orientation, the coalesced mass is controlled in some parts by one field and in other parts by the other; no intermediate resultant is ever formed.

The fields of the echinoderm egg.

To translate now the results of the sea urchin experiments into field terminology, one has merely to substitute the terms "animal field" and "vegetative field" for "animal processes" and "vegetative processes." By the time the sea urchin egg becomes accessible to experimentation, these two fields are already present, with the poles as dynamic centers (p. 226). Any given particle of the egg falls under the control of either one or the other field, depending on the relative strength of the fields in the given locality. The effects of the stronger field prevail. They lay down definite conditions for the subsequent development of the affected parts of the germ.

Apparently, these comprehensive fields become later subdivided into smaller fields, each of which is endowed with its own independent sphere of influence. This gradual breaking up of larger dynamic systems into smaller systems of progressively increasing specialization, restriction and independence is, as will be explained later, a fundamental character of development. Thus, as a result of the actions of the animal field, local fields for the formation of the stomodaeum, the ciliary bands, the apical tuft, etc., appear, while the vegetative field breaks up into an intestinal and skeletogenous field, the former continuing with its segregation into the various parts of the intestine, the coelomic derivatives, etc. *The fate of an individual cell depends upon the particular local field within which it happens to lie; this local field itself, however, has been segregated from an earlier and more generalized field of higher order.* Since this process of decentralization can be followed into much greater detail in higher forms which will be taken up later, we postpone its further discussion.

The field as expression of the collective character of development.

The field concept stresses the *dynamic* nature of developmental organization, as against the *static* character accorded to it in the old mosaic concept. Every phase of development is the result of *interactions between the material whole with its field properties on the one hand, and the material parts on the other.* Every germ must be viewed in this double light: as the originator and carrier of organizing activities, i.e., fields; and, at the same time, as an assembly of individual parts exposed and reacting to those field activities. This duality of viewpoint applies to fractions of the germ as well. A group of cells acts *collectively* through its field, but, at the same time, its constituent cells react *individually* to the field influences. For example, remember the case of micromeres transplanted into an animal half (example 38): first, collectively, they project a vegetative field into the surrounding animal material, and individually, they react to this field by forming skeleton, as called for in the vegetative field pattern.

Determination.

A cell of a sea urchin blastula has no definitive destination yet. At a later date, however, we find its assignment fixed. The event, or series of events, achieving the change from the indefinite to the definite condition has been called "*determination.*" When every part has received an irrevocable instruction which can lead it to a definite destination, organizing activity has done its part; ulterior development is a mere translation of those instructions into actual effects. Gradually the local differences of growth rates, movements, differentiations, etc., emerge as recognizable, manifest characters. Experience has taught us that *the determination of these characters dates back to an earlier stage than their actual appearance* (see p. 254 on isolation of organ rudiments). We can say, therefore, that parts which have started to develop *as equals* become gradually *determined* in various directions, which makes them *intrinsically unequal,* although this inequality, at first, remains latent and only in time becomes manifest and discernible. However, the distinction between latent and manifest differentiation cannot be fundamental. For, if two cells which were originally alike become "determined" to develop in different directions, we must assume that their physical and chemical constitutions have undergone divergent changes, even if there is no external criterion for this. One could almost call *determination "invisible differentiation."* It is to be hoped that in some future time application of sensitive physico-chemical tests will detect differences among cells which escape mere microscopic observation. Until then, one must keep in mind that the visible histological differentiation of a cell follows its "determination" with a certain lag.

Learning about *determination* as a fundamental trait of development, one becomes immediately curious about the manner of its operation. Numerous questions come to the mind, such as: Is determination a sudden and explosive event laying down the main features of development in one stroke, and for all parts simultaneously? Or is it a gradual process reaching different parts at different times? If the latter is true, is there a regular time order in which it affects the parts? Does it produce every specific detail at once, or does it operate by degrees leading from more general to more specific restrictions and instructions? Further, does determination embrace all the various component processes of which development consists (see Part One), such as growth, morphogenesis, histogenesis, etc., in a single unitary process, or does each component event have its own phase and fate of determination? Many of these questions will be answered below in the light of amphibian experiments. For a general orientation, however, the sea urchin egg may again serve as guide.

Determination of cleavage conditions.

The early cleavage stages of the sea urchin egg follow, as we have seen (Fig. 43), an absolutely definite pattern. This definiteness contrasts sharply with the essential indeterminacy of these same blastomeres with regard to their future differentiation. It seems, therefore, that determination of one character of development does not necessarily imply determination of all others; for, obviously the determination of the cleavage pattern precedes that of other characters by a long time. Of course, one could object that cleavage conditions might possibly be an innate and predetermined character of the egg. This objection cannot be upheld, however, in view of the following experiments.

As can be seen from Figure 43, the most striking inequality in the cleavage pattern of a sea urchin egg appears during the fourth cleavage step (transition from the eight- to the sixteen-cell stage). While the upper four cells divide into mesomeres of equal size, the lower four cells divide each into a large macromere and a smaller micromere. This cleavage pattern is conditioned by differences between the upper and the lower cells of the eight-cell stage regarding the position and orientation of the mitotic spindles. The question thus arises as to when these specific differences were inaugurated in the protoplasm of the blastomeres.

Example 45.

In order to decide this issue, eggs were divided meridionally at different times, and the cleavage pattern of the halves was recorded.[1] Let us first consider the result of transection after the third cleavage step, i.e., in the eight-cell stage. After the operation the half germ consists of two animal and two vegetative cells. These cells behave during the next cleavage step precisely as if they were still in connection with the removed half. That is, the two animal cells divide meridionally into four equal mesomeres, while the two vegetative cells yield two macromeres and two micromeres. We shall call this type of cleavage pattern a "*half*" pattern. Let us then go back one step and separate one half of a four-cell stage. During the next cleavage step, which is the third, the isolated two blastomeres divide equatorially, just as they do in the third cleavage step of an intact egg. The resulting four cells, which correspond to a lateral half of an eight-cell stage, during the next step divide unequally — the upper pair into mesomeres and the lower pair into macromeres and micromeres. Thus we obtain again a "half" cleavage pattern. If we go further back and separate a blastomere of the two-cell stage, the result is essentially similar, cleavage proceeds according to the "half" pattern. Consequently, the conditions which lead to the unequal sixteen-cell stage are already fixed as early as in the two-cell stage. For this reason, the experiments were carried back into still earlier, pre-segmentation, stages. If half of an unfertilized egg is separated and fertilized, it cleaves like a normal, whole egg. That is, after reaching the stage of four cells, it does not cleave unequally to produce one-half of a sixteen-cell stage, as did the older halves, but it gives rise to eight equal cells which only in the next succeeding step divide unequally, yielding a whole sixteen-cell stage with eight mesomeres, four macromeres, and four micromeres. This proves that the halved mass had received a *total* pattern of cleavage conditions.

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[1] *Hörstadius*, 1928. See also *Harnly*, 1926.

Individual portions of the egg prior to fertilization are, therefore, not yet in possession of their final cleavage plan. In the two-cell stage, however, they are. Consequently, *the cleavage pattern must have become fixed in the egg material at some time between fertilization and the appearance of the first furrow.* In fact, by dividing eggs at various times between these two events the progressive fixation can be followed.

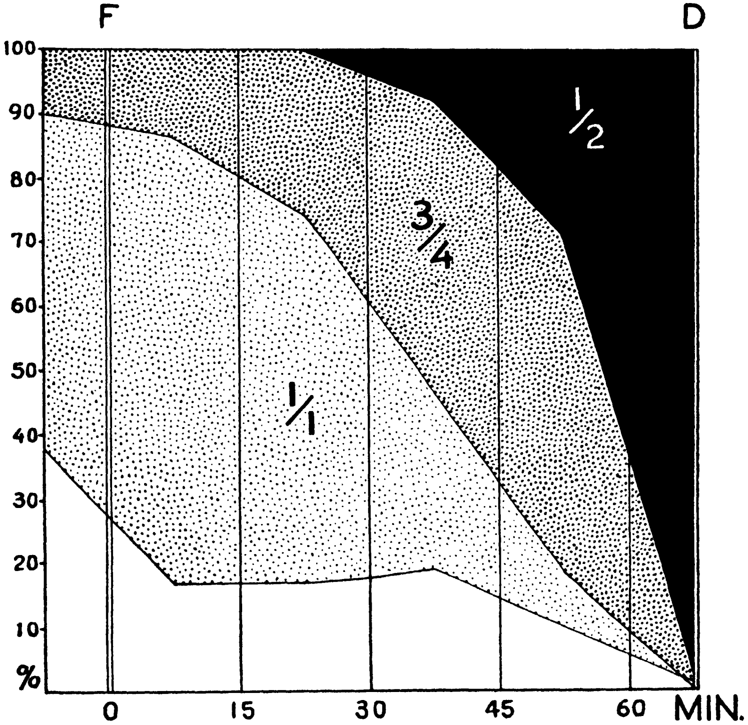

FIG. 57. Gradual fixation of cleavage conditions in the fertilized sea urchin egg. (Constructed from data of *Hörstadius*) Eggs were bisected at 15 minute intervals between fertilization (F) and the appearance of the first furrow (D), and the percentage of fragments cleaving like *whole* eggs (1/1, lightly stippled), *half* eggs (1/2, black), *intermediate* (3/4, darkly stippled), and *aberrant* (white), was recorded. Note the steady decline of the percentage of "whole" cleavage patterns and the corresponding increase of fragmentary cleavage patterns, as the interval between fertilization and transection is increased.

It takes about one hour after fertilization for the first furrow to occur. If the fate of egg halves cut at various times after fertilization is watched, it is found that the later the fragments are cut, the less likely are they to show the "total" cleavage pattern. At the same measure as the percentage of "total" cleavage falls off, the percentage of "half" cleavage increases, with a certain proportion displaying an intermediate pattern. Figure 57 illustrates this situation.

Cleavage and differentiation are determined independently.

These results show plainly that the subsequent cleavage behavior of individual regions of the undivided egg is by no means an unalterable primordial property of these regions, but is gradually acquired after fertilization. The critical time coincides with the preparations for the first cleavage step. After that everything is fixed, so far as the cleavage pattern is concerned. It hardly needs repeating that with regard to differentiation and organization, however, the individual portions of the germ have incurred no final obligations. So, we have confirmed that *determination of one event of development does not necessarily imply the determination of other developmental events.*

These observations, at the same time, corroborate our contention, explained above, that the cleavage pattern is in itself no factor in the production of the pattern of organization and differentiation of the embryo. For, even those meridional halves which have cleaved according to a "half" pattern give rise to complete and proportionate larvae; the defective cleavage pattern reflects in no way upon the later organization of the individual.

Determination of size in the sea urchin.

Although larvae developing from egg fragments can be morphologically complete, of typical proportions, and normal in histological regards, they still have one deficiency, namely, they are *subnormal in size.*

Example 46.[1]

> When the cells of larvae reared from meridional egg halves are measured and counted, it is found that their size is approximately that of corresponding cells in a normal larva of comparable age, whereas their number is reduced to approximately half the normal number. A larva raised from a halved egg compares to a normal larva, as a small house compares to a large house, if both are built from bricks of identical size. In the dwarf larvae the size of the individual cells is relatively too large in proportion to the size of the whole body.

Again we encounter here two developmental results which must have been determined independently: *cell size* and *body size*. Their relation seems to be this: Since no appreciable growth takes place during segmentation (p. 78), larvae hatching from half eggs must be smaller than normal larvae. Larval size, thus, depends on the amount of material available for development. The lower size limit of cells on the other hand, is definitely fixed as a species-specific character.[2] It is not surprising, therefore, that cell size in the reduced larvae has failed to become adjusted to the diminished proportions.

In the present case this lack of adjustment is not serious. But it shows us the fundamental source of discrepancies that can be fatal for development. Whenever two originally independent developmental events whose later cooperation is indispensable for development are brought out of tune by genetic or environmental accidents, there is danger. When they fail to meet at the proper time, development may be arrested or upset. Concrete examples of this kind will be furnished later (p. 479 f.). They all emphasize the importance of correct spacing and timing of the various determinative events for the smoothness and orderliness of development. The developing organism is not unlike an assembly plant whose effective operation hinges on the precision with which the individual assembly lines feed in the parts at intervals well calculated in advance.

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[1] *Morgan*, 1896.

[2] This is true only provided the nucleus contains the full set of chromosomes characteristic of the species.

ANALYSIS OF AMPHIBIAN DEVELOPMENT

Potencies

Although the echinoderm egg deserves a large share of the credit for the modern development of Analytical Embryology, it is doubtful whether even the most intense study of this single object could ever have brought us such specific knowledge about fundamentals of development as we now possess. For, the variety and specialization of organs and tissues are fairly modest in an echinoderm larva; at any event, they are inferior to those of even the simplest vertebrate. Under these circumstances it has been a veritable boon to our science that germs of vertebrates, especially amphibians, have proved to be just as accessible to experimentation as the echinoderm eggs. The experiments have also been extended to the germs of *birds*, *fishes*, and *mammals* among the vertebrates, and to *insects* among the arthropods, but none of this work has as yet attained such prominence as that done on *amphibians*. For this reason, we shall let our further discussion be guided by the results obtained on amphibian germs during more than three decades of persistent, painstaking, and well-planned experimentation.[1]

Regulation in amphibian eggs.

The basic fact about the sea urchin egg has been that it does not begin its development as a rigid mosaic. This fact likewise holds true for the eggs of *amphibians*. Evidence for this contention can be found in the fact that twins (examples 15, 35) can be obtained from a single normal egg. Even germs transected as late as in the blastula stage can still give rise to a whole embryo from each half.[2] Again, when half of an egg forms a whole embryo, this is not accomplished by secondary regeneration (so-called postgeneration) whereby each half would continue to develop as a half and would supplement the missing half only secondarily, by later growth processes. If the egg is split at a sufficiently early period, morphogenesis of either half proceeds from the beginning according to a *whole* rather than a *half* pattern. This entails considerable deviations from their regular prospective fates for all parts, which, in turn, proves decisively that the parts of an amphibian egg, at the onset of development, cannot possibly have definitive detailed destinations. The assumption of preformed reserve mechanisms in each half providing for whole development after accidental splitting can be invalidated by the same argument as has been put forth in the analogous case of the echinoderm egg, namely, the fact that early *fusion of two complete eggs* can result in the formation of a *single uniform embryo of double size.* One could not reasonably contend that a normal egg might contain a preformed emergency mechanism for the production of a giant half-larva; for, the occurrence of such an emergency in nature is unthinkable.

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[1] *Spemann* (1938) has furnished a thoughtful review of these studies in which he and his school have taken a major share.

[2] Provided the cut has divided the germ symmetrically.

Example 47.[1]

Uniform embryos from fused germs.

Two whole amphibian eggs can be merged into one by cross-uniting the two-cell stages in the manner illustrated in Figure 58. If the two partners are fused in a certain orientation (about this point see further below), *a uniform embryo of normal proportions but of giant dimensions develops.* Figure 59 shows such an embryo together with a normal control embryo of the same age. Its double size is clear proof that the materials of both eggs have actually been used in its formation.

Owing to its "regulative" capacity, the amphibian egg must thus be included in the same developmental class as the echinoderm egg. In their early stages both come close

[1] *Mangold and Seidel,* 1927.

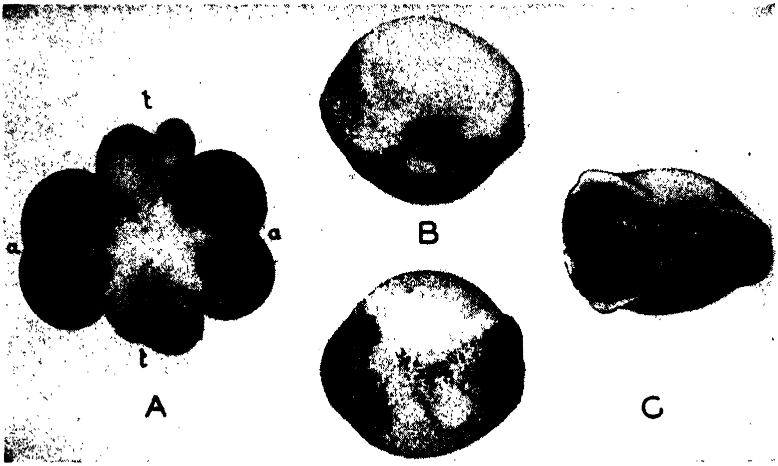

FIG. 58. Crosswise fusion of two newt germs of different species (*Triton taeniatus* and *T. alpestris*), effected in the 2-cell stage. (From *Mangold* and *Seidel*, 1927) × 10.5.

A, Cleavage division following the fusion. The new furrows cause a shamrock-shaped outline. The *alpestris* blastomeres (a) are larger and more heavily pigmented than the *taeniatus* blastomeres (t).

B, Composite germ in the late gastrula stage, posterior and anterior views. The derivation of the cells is still clearly recognizable by the sharp differences of pigmentation.

C, Neurula stage.

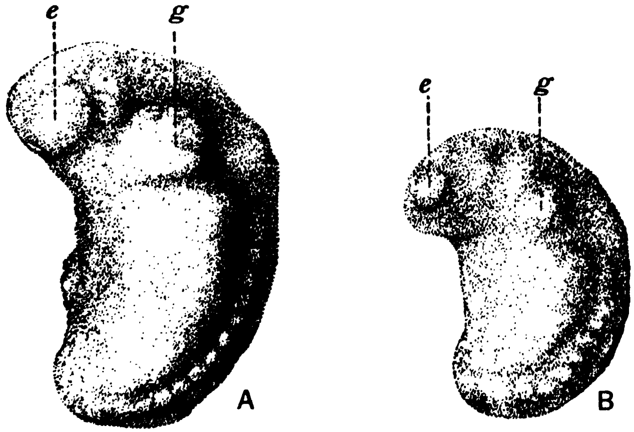

FIG. 59. **Development of a single giant embryo from two eggs of *Triton taeniatus* fused in the manner of Fig. 58. (From *Mangold* and *Seidel*, 1927) This case corresponds to combination D of Fig. 73. A, Giant fusion product. B, Normal control embryo of identical age. *e*, Eye vesicle. *g*, Gill rudiment. × 21.**

to being harmonious-equipotential systems. We remember from the sea urchin experiments what this designation implies: first, that the parts have no definite developmental assignments; second, that organizing activities of definite order deal out a harmonious pattern of assignments; third, that the individual parts can adequately respond to these assignments and translate them into manifest differentiations. Experiments on amphibian germs have enormously expanded our knowledge of these points.[1] The results can be condensed into the following account.

The differentiation potencies of blastula cells.

1. *The individual cells composing the amphibian blastula are still in possession of numerous differentiation potencies;* their fate is still undecided and they can exhibit striking versatility. Transplantation is the standard method of testing whether cells do or do not possess irrevocable trends of differentiation. From watching the development of countless numbers of eggs under normal conditions, aided by the vital staining method (Fig. 60), one knows the *prospective*

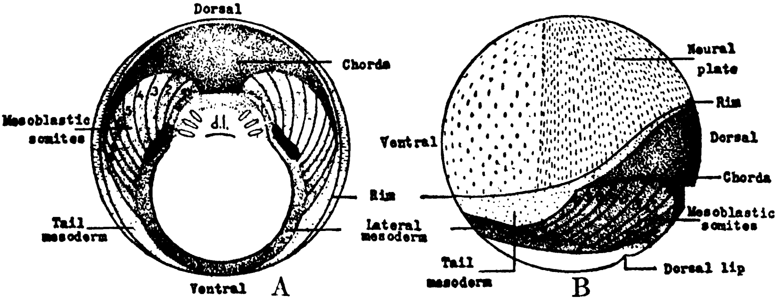

FIG. 60. Diagrammatic representation of the presumptive fate of various areas of the urodele blastula, as determined by the tracing of vital stain marks into the embryo. (After *Vogt* from *McEwen*) The chart merely indicates what becomes of different cell groups in the course of normal undisturbed development.

A, View from vegetative pole. Entoderm white.

B, View from left side.

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[1] A brief survey of the work on organization of amphibian development can be found in *Weiss*, 1935b.

fate of any given cell group of the blastula.[1] To test its *prospective potency*, one must remove it from the conditions under which it would realize its normal fate and incorporate it in regions of divergent destination.

Example 48.[2]

Interchangeability of cells within the ectoderm.

> A small area of ectoderm is exchanged between two germs which are both just entering gastrulation. The fragments are chosen of identical size so that they fit into each other's places. One is taken from the region of the later brain, and the other from a region which is to form belly skin (Fig. 61). The two germs are selected from two different species of sufficiently distinctive pigmentation in order to make possible later identification of the transplanted cells (see p. 130). If the transplants continued to develop according to their original prospective fate, one would have to expect that one of the resulting individuals would carry an island of skin in the middle of its brain, and the other a fragment of brain in its belly. In reality, however, both embryos are perfectly normal except that the brain of one, and the skin of the other contain each a streak of cells of different pigmentation (Fig. 61B$_2$, C). If it were not for their distinctive coloration, it would barely be possible to recognize the transplants, so smoothly have they become incorporated into their new surroundings, and so harmoniously have they been utilized in the building processes of their new locations.

Both transplants have *complied with the formative tendencies of their new surroundings.* This proves, first, that these particular cell groups had been devoid of a definitive developmental plan of their own at the stage of transplantation, and,

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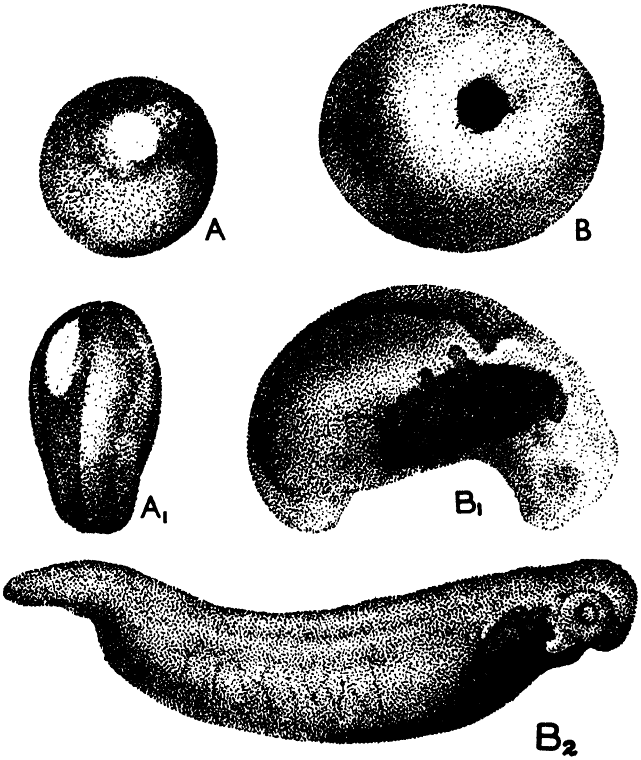
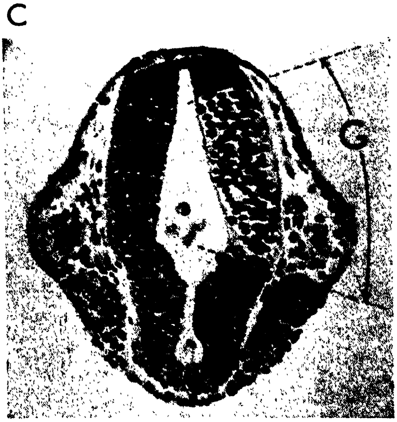
[1] Our present precise information concerning the fate of various sectors of the blastula during subsequent development goes largely to the credit of the observations on vitally stained germs by *Vogt* (1925, 1929) and his school. Anurans and urodeles are fundamentally alike, so far as the general distribution of presumptive organ related districts is concerned; the relative extent of different areas varies, however, between both groups. It must be stressed that the sharply outlined sectors of the diagram (Fig. 60) are, of course, not distinguishable or differentiated in the living object; the diagram offers merely a backward projection upon the blastula of the known future fate of its various portions.

[2] *Spemann*, 1921.





A, B, Early gastrulae of *Triton taeniatus* (pigmented) and *T. cristatus* (light) after the interchange of a patch of ectoderm. × 16.

A_1, Neurula developed from A. The graft, recognizable by its lack of pigment, lies in the neural plate. × 16.

B_1, B_2, Later stages of the germ B. The pigmented graft has formed epidermis. × 16.

C, Cross section through a later stage of embryo A_1. A sector (G) of the host's brain is composed of cells derived from the graft. × 75.

FIG. 61. Reciprocal heteroplastic transplantation of germ fragments. (From *Spemann*, 1938)

secondly, that they were acted upon in their new locations by organizing factors which gave them a definite direction in conformity with what was going on in that region. It will be noted that in this experiment each fragment had been transposed within its own germ layer, the ectoderm. That the versatility of cells is not confined, however, to these limits is shown by the following experiment.

Example 49.[1]

Interchangeability of cells between different germ layers.

Fragments of *ectoderm* taken from pre-gastrulation stages can be placed into such positions that they will become incorporated into the invaginating *mesoderm* during the course of gastrulation. If this happens, the transplanted ectodermal cells take part in the formation of the mesodermal derivatives of the location; cells which would have normally produced some brain or skin tissue are found now actively engaged in the building up of musculature, notochord, or pronephros. Incorporated into the *entoderm* they have been seen to produce part of the lining of the primitive gut.[2]

In conclusion, we must attribute to the cells of the *ectoderm* of an early gastrula the *full potency to differentiate in harmony with the morphogenetic and histogenetic trends of any locality of the germ.*

If presumptive *mesoderm* of an early gastrula is incorporated into *ectoderm,* one occasionally obtains analogous results. That is, the mesodermal cells may desist from their original task, which would have been to form either notochord or musculature or skeleton or pronephros, and simply become part of the covering epidermis of the body.[1] In most cases, however, it is difficult to keep mesodermal transplants in the surface of the germ, owing to their intrinsic tendency to roll in and to disappear from the surface, which, of course, removes them from the domain of the ectoderm. Cells from the median area of the invaginating mesoderm are the least conforming of all. Grafted into whatever location, they continue to invaginate and to give rise to mesodermal derivatives.[1] As we shall see later, this does in no wise mean that their *individual* cells are less compliant. Only we cannot transplant individual cells; so we must always graft larger areas, and these often exert their own organizing influences upon their constituent cells, thus preventing the latter from joining other formations going on in the vicinity. As a matter of fact, there is decisive proof to show that individual mesoderm cells in the gastrula stage are not yet definitively determined and can substitute for each other freely throughout the mesoderm.[2] Furthermore, there is definite evidence from other types of experiments that individual cells of the prospective mesoderm can take part in the production of practically all ectodermal derivatives, such as nervous tissue, epidermis, teeth, and ectodermal glands.[3]

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[1] *Mangold,* 1923.

[2] This interchangeability among cells from different germ layers has been confirmed repeatedly; for the production of entodermal structures by ectodermal cells, see *Holtfreter,* 1936, on page 501. Also *Raven,* 1935.

It has not yet been possible to ascertain potencies of comparable latitude for *entodermal* cells. In amphibians, the reason seems to be more of a technical nature inasmuch as it is difficult to visualize how it should be possible, from purely mechanical considerations, for the huge, yolk-laden entoderm cells to coöperate with any of the other, small-celled, germ layers. In birds, however, in which otherwise the situation is very similar to that found in the amphibians, entoderm cells, too, have been demonstrated to possess wider potencies than they actually realize in the course of normal development.[4]

Innate cell characters.

2. While cells have thus not yet received definite assignments concerning their future *correlative* and *histogenetic*

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[1] *Lewis*, 1907; *Spemann and H. Mangold*, 1924.

[2] *Holtfreter*, 1931a; and many others.

[3] *Holtfreter*, 1936.

[4] Indications of interchangeability of entoderm cells in the chick were reported by *Rudnick and Rawles*, 1937.

development as late as in the gastrula stage, *others of their characters are found to be fixed from earlier stages on.* Some of these are primordial characters, ingrained properties specific for the particular species of which the cell is a descendant part. One such character is *nuclear size* which remains unaltered even if the cell is forced to live in the midst of cells of another species with smaller or larger nuclei.[1] It is for this reason that we can use nuclear size in transplants as a convenient means of tracing cells into their later fate (Fig. 17, p. 131).

The degree of primary *pigmentation* is another fixed character of the cell. As can be seen from Figures 58 and 61, cells are colorfast, so to speak, however plastic they may be in other regards. Another property persistently retained is the *biochemical differential.* Reference to these biochemical differentials among species was made above (p. 166). It is true that in very early embryonic stages parts can be exchanged between germs of two species whose adult tissues would not tolerate each other.[2] But the conflict between tissues of biochemically different constitution which have shown mutual tolerance in earlier stages may yet show up at some later period. Under these circumstances transplants which have become morphogenetically assimilated into the host are afterwards destroyed and eliminated,[3] or can even sometimes reciprocally poison the host body.[4]

Innate developmental rate.

Finally, an important character which each cell has directly inherited from the egg is what we might call its *tempo* of operation. Every cell of a given species seems to possess a definite time standard which serves as a regulative norm for all its vital activities. That is, a cell is by constitution slower or faster and manifests this fundamental time character in all its later performances. Thus, when organizing influences of a host body make a strange cell do team work in the local building processes, they cannot apparently determine the absolute speed of its proliferation and differentiation but can only effect a relative acceleration or retardation of the basic rate with which the cell is endowed. Exposed to the same field, material of a species of basically higher rate grows faster than material from a species of basically lower rate. Translated into the terms of our description of growth in Part One (p. 22), this means that each individual cell inherits from its species an *innate growth potential*, that is, a predisposition for a certain basic rate of growth and division, which yields a wide variety of actual growth rates, depending upon the growth-promoting or growth-depressing *circumstantials* of the surrounding body.

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[1] *Raven*, 1935.

[2] The exchange of embryonic tissue has been successful between amphibians and teleosts (*Oppenheimer*, 1936c) as well as between birds and mammals (*Nicholas and Rudnick*, 1933; *Waddington*, 1934) which are incompatible in the adult stage.

[3] *Filatow*, 1925.

[4] *Bytinski-Salz*, 1929; *Twitty*, 1935.

This situation was discovered in the course of the experiments described in example 48. There it was found that the transplanted ectoderm which had come to cover the gill region of the host larva and, accordingly, had been utilized in the building up of the gills of that side had grown and differentiated faster than the host's own gills of the opposite side. Evidently, *the grafted cells have complied with the local organizing demands, but have done so with their own innate tempo.*[1] As we shall see later, a cell derives not only its basic growth rate but also its specific potencies for differentiation from its permanent inherited endowment rather than from outside sources.

These examples may suffice to show how meaningless it is to call a cell simply "determined" or "undetermined";[2] there are certain characters which form a permanent and inflexible endowment of the cell and which are, therefore, determined from the very start, while others receive their determination later at various periods of embryogeny.

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[1] *Spemann*, 1921; *Rotmann*, 1931.

[2] Compare the lucid criticism of the term "determination" by *Harrison*, 1933.

Decline of cellular potency.

3. *During gastrulation parts of the germ which up to then have been freely interchangeable lose some of their versatility.* The first indication of this decline of potency is seen in a greater resistance of the cells against morphogenetic demands other than those leading to their prospective fate. Even at this stage, presumptive epidermis can still be turned into brain and vice versa, but the reaction is decidedly *delayed.*[1] One obtains the impression that the cells had already started certain preliminary differentiations whose traces had to be undone first before there could be any new differentiation in accordance with the changed locality.

Consolidation of self-differentiating power.

4. *By the end of gastrulation the fate of cellular districts,* if not of individual cells, *has become definite and final.* This fact manifests itself in two ways: in the self-differentiating capacity of such fragments when transplanted, and in their failure to yield to organizing demands coming from new locations.

Example 50.[2]

> We repeat the experiment of example 48 except that we use germs of older age. If the exchange is effected in the neurula stage or still later, the result is fundamentally different from the one obtained in example 48. Now, the prospective brain develops *according to its original prospective fate* and produces an island of brain tissue in the midst of a strange vicinity (Fig. 62). Similarly, the prospective skin portion forms an insert of epidermis among a mass of nervous tissue.

This result shows that by the time the germ has reached the neurula stage, its several portions have become capable of pursuing definite courses of development independently.

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[1] *Lehmann,* 1929.

[2] *Spemann,* 1921; cf. also *Mangold,* 1929c.

They not only possess the necessary directives, but also sufficient strength to put these into effect, even in the face of entirely incongruous surroundings. They continue approximately along the same lines as if they had been left in their old locations. This result is, of course, in full agreement with the isolation experiments described in examples 27 to 30, proving the self-differentiating capacity of later stages. But there is this difference: By contrasting examples 48 and 50, we now have the definite proof that *self-differentiating capacity is a secondarily acquired rather than a primordial property.*

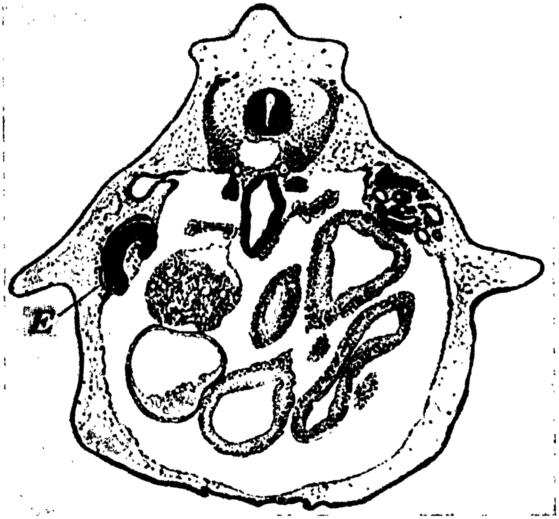

FIG. 62. Self-differentiation of grafted brain portion. (From *Spemann*) Presumptive eye region of the medullary plate of a neurula of the anuran, *Bombinator pachypus*, transplanted to the body flank, has differentiated into an eye (*E*).

Emancipation of germinal districts.

5. Just how the various areas of the germ acquire their specific partial assignments for further development, does not become evident from these experiments. But the fact is clear. *The germ emerges from gastrulation with a decen-*

tralized constitution; its various parts have obtained a certain measure of autonomy and definitely circumscribed powers. Local autonomous centers of this sort fit the definition of *fields* (p. 293). Once these local fields have been established, the organism as a whole, while still exerting some *general* control, has lost its *specific* grip on the individual parts. As we have seen, the residual control does not even go so far as to prevent very absurd formations, such as those described in example 50 and others to be described below. The late germ no longer has sufficient dynamic unity as to exert the regulative powers which were characteristic of the younger germ. Instead, various local fields have assumed local rule within their own rights. After that, fragments of the germ can no longer transform into whole individuals when raised in isolation, but produce incoherent parts of the body (examples 27 to 30).

The establishment of local autonomy in distinct areas of the germ may be called *emancipation.*[1] It connotes that different parts acquire some degree of self-rule and specific instructions as to how to apply it. The analogy refers to the political organization of human society, which, as has often been pointed out, is modeled after the pattern of organisms. Of course, the only advantage of such analogies is that they translate observations into more familiar language; no deeper insight into the mechanisms is thereby gained. But so long as the terms permit an adequate description of the principles involved, they seem passable.

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[1] This term (*Weiss*, 1935b), offered in translation of the German term "Autonomisation" (*Weiss*, 1926) might be fully replaced by the term "dynamic segregation" were it not for the fact that *Lillie* (1929) has spoken of "segregation" in a slightly different sense. Nevertheless, we shall use the terms "emancipation" and "segregation" synonymously, to denote the same phenomenon. Part of the phenomenon, namely, the purely quantitative aspect, is covered by the term "physiological isolation" introduced by *Child* (1929a). The term "individuation," suggested by *Waddington and Schmidt* (1933) is also fitting.

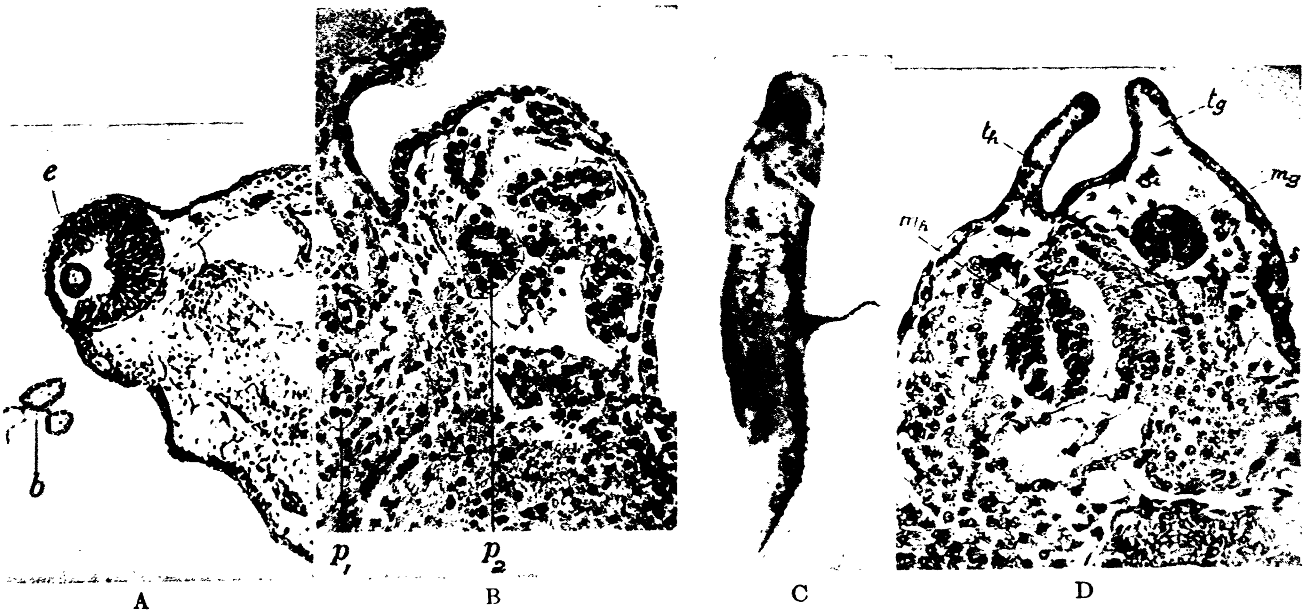

FIG. 63. Testing of the fields of the neurula by grafts of indifferent ectoderm. (From *Holtfreter*, 1933)

A, Gastrula ectoderm grafted to the head region has produced head structures; the section shows balancers (*b*) and eye (*e*) with lens.

B, Gastrula ectoderm grafted to the anterior trunk region has differentiated pronephros. p_1, Pronephric tubules of host. p_2, Pronephric tubules developed from the graft.

C, D, Gastrula ectoderm grafted to the posterior trunk region has produced a tail. C, view of the differentiated specimen. D, cross section through the larva at the base of the secondary tail. m_h, Neural tube of host. t_h, Dorsal fin of host. m_g, Neural tube of graft. t_g, Dorsal fin of graft. *s*, Somitic musculature of graft. Note the eccentricity of the lumen of m_g (see Fig. 112d).

Organization continues on smaller scale.

6. The local field districts emancipated during and after gastrulation are endowed with organizing power narrower in range than the one to which they owe their origin. *Each of the segregated field districts is in charge of a limited group of formations* which are its specialty, as it were, — eye, brain, nose, ear, gills, limb, etc. We can demonstrate the operation of these local fields by confronting them with young tissues which we know to possess proper responsiveness; according to examples 48 and 49, ectoderm of an early gastrula represents such tissue.

Example 51.[1]

Testing local fields by grafts of reactive cells.

Flaps of gastrula ectoderm were grafted to the flank of older embryos which were at or beyond the neurula stage. The transplanted cells healed in smoothly but, unlike example 48, they usually failed to participate in the structures of the host body whose differentiation was under way. They remained outsiders, so to speak, probably because of the considerable difference in age between donor and host. But they gave rise to complex supernumerary formations whose organ character could be readily identified. There were eyes, brain parts, noses, ears, muscles, pronephroi, tails, etc., as well as various combinations of these (Fig. 63). They formed large irregular masses bulging forth from the body wall, highly abnormal in their arrangement, morphology, and connections. Nevertheless, one definite element of order could be recognized, and this is of crucial importance: there was a *definite correlation between the types of organs differentiated from the grafts and the character of the host region in which they were located.* In the head region the transplants contained head organs, such as eyes, ears, nose, balancer (Fig. 63A); further back, in the neck region, ears and gills were most frequent; in the anterior region of the trunk pronephros and musculature were common (Fig. 63B); and in the posterior trunk region the transplants almost always developed into tails (Fig. 63C, D).

[1] *Holtfreter,* 1933b.

The overlap of fields.

On the whole, the character of these extra organs, thus, seems to be determined by local fields of the host body. At the same time it becomes evident, however, that *every one of these fields extends far beyond the actual limits of the corresponding host organ.* Transplants often gave rise to an eye or ear or gill or tail, even though they were separated from the normal eyes, ears, gills, or tail of the host embryo by some distance. Eyes have been seen to arise as far back as the ear region or even farther, and tails have been found as far anteriorly as the middle of the trunk. Nevertheless, when a large number of cases is surveyed, it is found that *the frequency of eye formation is greatest* in those transplants which lie *closest to the eye region,* the *frequency of ear formation highest* in those *closest to the ear level,* and so forth. In other words, the normal eye district appears as a center of eye-forming activities, the normal ear district as a center of ear-forming activities, and so forth, and with increasing distance from any one of these centers the chance to obtain the corresponding organ declines rapidly. The result reminds one of the situation in the sea urchin. Just as there the capacity for animal and vegetative formations declined with increasing distance from the poles, so in the present case the intensity of organ formation falls off gradually from certain centers whose position is marked by the sites of the normal eye, ear, gill, tail, etc. Obviously, *the normal eye develops in the center of an eye field and the normal ear in the center of an ear field, but either of these fields extends far into the other's domain;* and a similar consideration holds for the fields of the gills, tail, and so forth.

This leads us to the same concept at which we have arrived from the sea urchin experiments; fields appear as dynamic entities whose activity reaches a maximum in a center and from there declines towards the periphery. In spite of the wide overlap of neighboring fields thus occasioned, there is no confusion among the resulting formations;

the explanation of this lies evidently in the principle of exclusivity (p. 99) according to which differentiating material cannot simultaneously follow divergent trends. A cell group lying in the area of overlap of two fields, unable to produce some sort of compromise structure, can obey only either one or the other. Consequently, it will have to be either eye or ear or gill, even though all three or more fields may be potentially present. The decision as to which organ will actually be formed depends solely on the relative strength of the various fields in the locality: the strongest will prevail. After the material has become engaged in the activities of the dominating field, this very fact precludes its turning into the course of any of the weaker fields.[1] The experiments of example 51 prove at the same time that the failure of the neurula transplants in example 50 to develop in accordance with their new surroundings could not have been due to lack of an organizing field in those surroundings, but must be ascribed to lack of responsiveness in the transplanted materials; for, younger transplants, as we see now, do comply with the local character of development, even when transplanted into a neurula.

The persistence of fields.

7. *Field activity does not exhaust itself* in its effects. For, example 51 has just shown that eye fields, ear fields, tail fields, etc., after having once determined the formation of the normal eyes, ears, and tails, can repeat the feat if they are provided with the necessary plastic materials. This occurrence also documents the stereotypism and "blindness" of field actions (see example 39 and p. 274). Only under normal conditions do the field activities produce a normal individual. When confronted with such abnormal situations as an experimenter can contrive, they keep turning out their standard product, irrespective of whether or not this suits the needs of the organism as a whole. They exhibit the same

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[1] It is unnecessary, therefore, to assume special inhibitory actions to account for the suppression of the unused potencies of pluripotent cells.

rigidity and lack of adjustability which the biologist has become used to associate with *instinctive behavior patterns.*

Although fields retain their effectiveness for some time after the period of their actual utility is over, they do not, for the most part, persist indefinitely; by and by, they subside. If the same experiments as were described in example 51 are repeated in progressively older stages, using increasingly older germs as hosts, the development of the test grafts becomes poorer and weaker.[1] In view of the ascertained omnipotence of the transplanted material, this decline of organization can only be due to a decline of the organizing powers of the host fields. Each type of field, however, seems to have its own time line of efflorescence and subsidence; there are certain local fields, which evidently are preserved well into late periods of life and there form the basis of regeneration (see p. 473).

The orderliness of emancipation.

8. *The dynamic parcellation* (emancipation) of the germ into local field districts with circumscribed assignments *occurs according to a regular time and space order.* Local fields appear at definite periods and in definite distributions. Considering the wide expansion of fields, one realizes that balance among organ formations can only be maintained if adjoining fields check one another. If, for instance, the eye field were for some time left without competition, it would claim as much material as it could cover, which, as we have seen, includes at least the whole head region (example 51). All available material thus being engaged in eye formation, competitive fields (ear, etc.) arising with delay would remain ineffective because there would be no responsive cells left upon which they could act. Orderly development, therefore, would be impossible unless several fields coexisted, each endowed with a definite *intensity* so that the monopolistic use by any single field of all available materials of the germ is precluded. This implies that local field districts

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[1] *Holtfreter*, 1933b.

receive, in addition to a definite *pattern* of organization, a definite amount of *energy* with which to put this pattern into effect.[1] Whether pattern and energy spring from the same source, will be discussed on a later occasion (p. 387). For the time being, we keep in mind that both are necessary to endow a part with self-differentiating capacity. It is the possession of these two properties — *field pattern* and *field energy* — that enables local field districts of a late germ to finish their local differentiations even in complete isolation from the rest of the germ (examples 28, 29, 30).

The formation of organ rudiments.

9. Once the material of a local field district has begun to react manifestly to the field influences and has assumed a definite structure, it has become an organ rudiment (pp. 67, 254). According to point (6), such a rudiment occupies only the central region of the field district. Consequently, *extirpation of an organ rudiment does not radically extirpate the corresponding field district.* The periphery of the latter is left behind. We remember that in a sea urchin egg, when the normal center of the vegetative field, i.e., the region of the micromeres, is extirpated, the remaining peripheral parts of the field district take over the task of organizing the development of vegetative organs (gut, skeleton, etc.), although with decreased intensity (examples 37, 40). Likewise, the extirpation of an organ rudiment (i.e., the focal area of the organ field district) is followed by regeneration of the same organ from the residual periphery of the district.

An ear rudiment, for instance, consists of an ectodermal thickening (so-called placode) in the posterior head region which invaginates and forms a vesicle (otocyst). When this circumscribed area is transplanted to a heterotopic position, it develops in accordance with its prospective fate into a complete ear. Yet, at the place of its extirpation another ear develops from material which has moved in from the vicinity to cover the wound.[1] The same is true after the removal of a heart rudiment as in example 29,[2] of a limb rudiment,[3] of a balancer rudiment,[4] a gill rudiment,[5] and so forth. Prerequisite for the regeneration is, first, that the materials replacing the removed tissue have not yet received determination in a direction different from the one to which they are subjected in their new location, and second, that the extirpation has remained confined to the central portion of the field district. A more extensive excision amounts to a radical ablation of the whole field district, which leaves the region empty and impotent in regard to that particular type of organization. An embryo whose eye district has been completely excised remains permanently eyeless (p. 256 and Fig. 69). Similarly, earless, limbless, heartless embryos can be obtained, if much larger areas than those which would actually become incorporated in the rudiments of ear, limb, or heart, respectively, are removed.

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[1] In this connection it is instructive to remember the case of the sea urchin: the correct proportions of the larva depend on the proper balance between the animal and vegetative fields, and any shift of this balance upsets the final proportions (p. 28).

Progress of Determination

Progressive determination.

10. These studies leave no doubt that the first organizing effects of the germ consist of *imparting further organizing power upon the emancipated districts* rather than of calling forth final differentiations in the cells constituting those districts. This answers one of our questions (p. 297) concerning the progress of determination. *Determination advances by degrees from the more general to the more specific,* rather than in a sudden explosive act. Once established as such, each field district has become, so to speak, a little germ of its own inside the larger germ of which it now forms a discrete part. It has become a germ in the sense that it is

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[1] *Kaan,* 1926.

[2] *Ekman,* 1925; *Copenhaver,* 1926.

[3] *Harrison,* 1918.

[4] *Harrison,* 1925.

[5] *Ekman,* 1922.

itself now a sort of harmonious-equipotential system on a smaller scale, after the germ as a whole has long ceased to be one. Although certain regions have definitely and irrevocably acquired the destination to become eye or ear or limb or tail, their constituent elements are still free from final commitments. Here we encounter again the contrast between cell element and cell group. A whole *group* of cells has become sorted out for a circumscribed task (eye, ear, etc.), but what special role the *individual* cells are to play in the subsequent performance has not been determined at the same time; this is left for later activities of the emancipated partial system itself to decide. In order to test the "harmonious-equipotentiality" of organ rudiments, one proceeds in the same manner as if one were testing whole germs; that is, one interferes with the integrity of the rudiment and determines whether subsequent development is defective or harmonious.

Example 52.

The eye is determined, but its cells are not.

An eye rudiment capable of self-differentiation (see example 28) is transplanted close to a normal eye rudiment and merged with it. Out of this combination a *single harmoniously built*, although oversized, eye can develop provided some attention is given to correct orientation of the graft (Fig. 64A).[1] We remember that parallelism between the axes has also been a prerequisite for the development of a single individual from two fused sea urchin eggs (example 33). In other cases the internal structure of the eye shows signs of the composite origin (Fig. 64B).

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[1] *Pasquini*, 1927; *Detwiler*, 1929a. The fusion of double eye rudiments into a common median eye in cyclopia (Figs. 21 and 107; p. 483) furnishes additional evidence. Inasmuch as there is no detailed prelocalization of the elements of either eye at this early phase of development, it may be rightly questioned whether the old argument of a double versus single origin of the eyes (*Fischel*, 1921, against *Stockard*, 1910) has not lost its significance.

It is clear that in these cases of total or partial fusion the individual cells were disposed and differentiated in a different manner than normally. Therefore, although both eye rudiments as collective units were definitely marked for the formation of eyes, and nothing else, their constituent cell individuals were apparently still pluripotent to the extent that they could undergo any special differentiation within these limits; they could still become ganglion cells or pigment cells or sensory cells or Müller's fibers, etc. (see p. 86 and Fig. 12). That the resulting eye has typical organization, is due to the fact that the fusion of two organ field districts in proper orientation, according to point (7) on page 294, yields a single resultant field. More direct evidence of the interchangeability of the materials within the eye field is furnished by the following experiment.

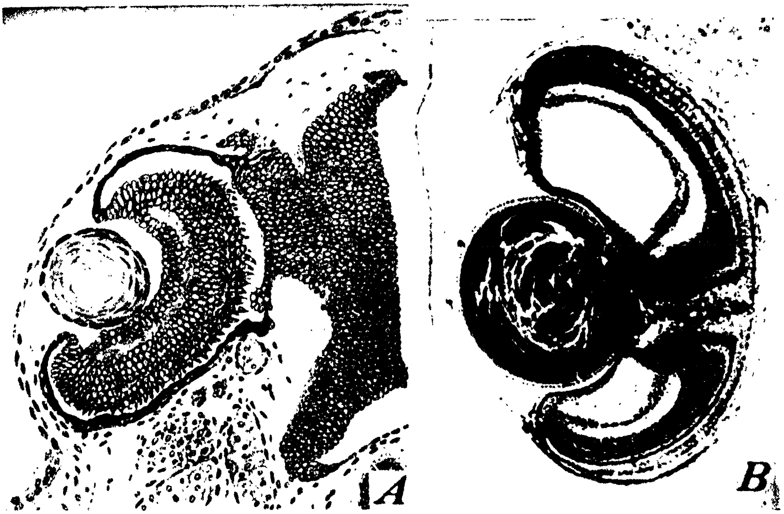

FIG. 64. Fusion of two eyes into one.
A, Single eye developed after complete fusion of two embryonic eye rudiments in the urodele, *Pleurodeles*. (From *Pasquini*, 1927) × 100.
B, Incomplete fusion of two eye rudiments in the frog, *Rana fusca*, has resulted in an externally uniform eye with single lens but internal subdivision. (From *Detwiler*, 1929) × 42.

Example 53.

The first morphogenetic event in the eye field is the formation of the eye vesicle, immediately followed by thickening and invagination of its lateral wall which thus becomes marked off as optic layer (retina proper) against the much thinner mesial wall which becomes the pigmented layer (tapetum) of the optic cup (Fig. 11). But, even though these two layers are distinctly different in appearance, their constituent cells have not yet become irrevocably fixed in either the retinal or the tapetal course. To demonstrate this fact, a fragment of the tapetal layer is carefully freed from adhering cells and then transplanted under the skin in the head region. Although devoid of presumptive retinal cells, such a graft transforms into a *proportionate double-walled optic cup*, with pigmented epithelium on the outside and a typical layer of high retinal cells on the inside[1] (Fig. 65). Thus, part of the prospective pigment cells of the tapetum have assumed the character of cells of the optic layer. Whether retinal cells in a reciprocal experiment would be able to restore

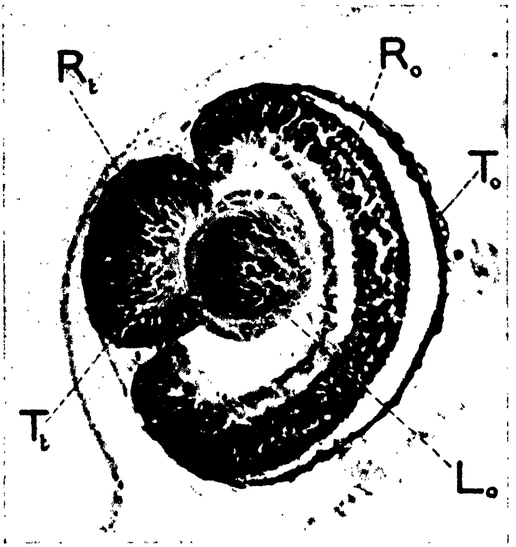

FIG. 65. Eye-forming potency of the outer layer of the embryonic eye cup. (From *Dragomirow*, 1932) A fragment of embryonic tapetum of the anuran, *Pelobates fuscus*, grafted to the front of the eye of a slightly younger host has produced a little eye cup with both tapetum (T_t) and retina (R_t). R_o, Retina of host eye. T_o, Tapetum of host eye. L_o, Lens of host eye. × 150.

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[1] *Dragomirow*, 1932.

the missing tapetal layer of the cup, has not been definitely ascertained;[1] but even if the regulation works only in one direction, the result proves that the destination of the *individual cells* composing the eye field district at this stage is much less definitely settled than is the fate of the *district as a whole.*

Equipotentiality within the heart rudiment.

Other organ rudiments behave similarly. The *heart* of a vertebrate is of bilateral origin. In the amphibian embryo the prospective heart-forming material lies in the two lateral mesoderm plates. Their advancing free borders meet in the ventral mid-line and merge, and it is in this region that the heart is formed (Fig. 66). As we have described earlier (example 29), the prospective heart district, when explanted in the tail-bud stage, forms a pulsating heart with the typical four chambers. This result proves that by that time the

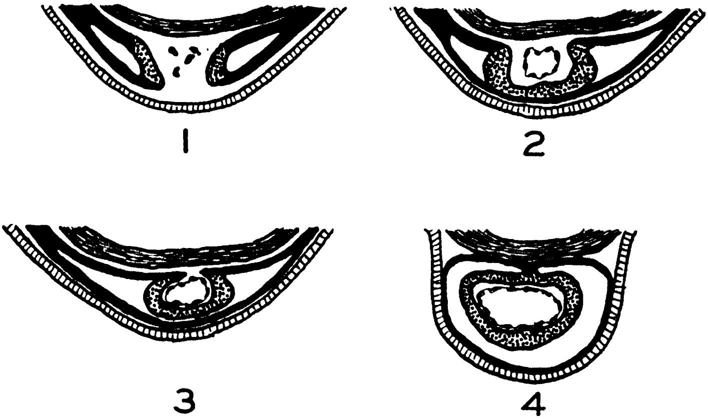

FIG. 66. Four stages in the development of the amphibian heart. (Diagrammatic after *McEwen*)

1, Downgrowth of the free borders of the bilateral mesoderm toward the ventral mid-line.
2, Ventral fusion of mesoderm.
3, Fusion of lateral pericardial cavities.
4, Advanced stage with heart suspended in pericardial cavity.
black . . . Pericardial mesoderm. stippled . . . Myocardial mesoderm.

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[1] The formation of pigment in isolated fragments of the optic layer has been observed in the chick (*Alexander*, 1937; *Dorris*, 1938).

heart field has become localized. Thereafter, when tested for equipotentiality, this heart field district behaves as follows:

Example 54.[1]

> The ventral fusion of the two free rims of the mesoderm folds (Fig. 66, 1) can be obstructed by interposing a mechanical block of strange tissue; the same result can be obtained by a gaping incision. Subsequently, each of the lateral halves of the heart rudiment thus prevented from combining, forms a separate heart. Such embryos have *two complete hearts*, one on the left and one on the right side, instead of a single median heart (Fig. 67).

One must conclude that either of the lateral plates contains a complete heart field and that the usual process of forming a single heart is comparable to cyclopia, in that the two lateral field districts merge into one, just as in cyclopia two lateral eye rudiments fuse into a single median eye. Although both halves are "heart-bound," the detailed fate of their cells is still in suspense. The individual cells take different courses, depending on whether there is separation (example 54) or fusion (normal development). Consequently, *the establishment of a localized organ field antedates the fixing of a definitive course for the individual cells within the field.* In the heart this condition lasts even beyond the stage of fusion, because if one splits the heart rudiment only after

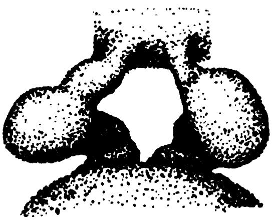

FIG. 67. Twinning of heart in the grass frog, *Rana fusca*. (From *Ekman*, 1929) In the early neurula stage a strip of branchial mesoderm was transplanted to the ventral mid-line of the body so as to block the median fusion of the bilateral heart rudiments. Result, 4 days after the operation: Two complete separate hearts have formed, both with circulation. Note the inverse asymmetry (situs inversus) of the right (in the picture, left) heart. × 66.

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[1] *Ekman*, 1925; *Copenhaver*, 1926.

the two portions have come together, each half can still give rise to a complete heart. By repeating this operation, as many as five separate hearts have been obtained from a single heart primordium.[1] Conversely, if an entire heart primordium is grafted on top of a normal already fused primordium, both can combine in the production of a large single heart.[2] As in the case of the eye (example 53), so the cells within the heart field district are still interchangeable at a time when the heart has already undergone its first specific morphological transformations.

Example 55.[3]

When the heart tube forms it is surrounded at some distance by an outer layer of mesoderm (Fig. 66) which normally becomes the lining of the pericardial cavity (pericardium), while the inner layer gives rise to the musculature of the heart proper (myocardium). It is possible in this stage to excise one lateral half, rotate it so that the outer layer assumes the position of the inner layer, and vice versa, and have the inverted half fuse with the normal half. If each half proceeded toward its original destination, an abnormal heart would result with one half turned inside out. Actually, however, *a normal harmonious heart develops* in which the presumptive pericardium and the presumptive myocardium of the reversed portion have traded not only places but also roles.

Equipotentiality within the limb bud.

Numerous experiments have been performed to test the harmonious-equipotential character of the *limb* rudiment in urodele amphibians. As a distinctly segregated area the fore limb field district has been traced back as far as the gastrula stage.[4] It then centers around some point in the somatic portion of each lateral plate. Transplanted to a heterotopic position, this district *self-differentiates into a limb.* Again, as in other instances of this kind (p. 320), the limb rudiment includes only the central portion of the limb field district, and its removal, therefore, does not preclude the formation of a limb from residual material moving into the gap from the vicinity. At a time, however, when the limb field district already possesses full self-differentiating capacity, the individual cells of which it is composed can still be substituted for each other freely; that is, although the fate of the whole cell group has been fixed, that of its individual members is still undecided. This may be concluded from experiments of the following kind.

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[1] *Ekman*, 1927.

[2] *Copenhaver*, 1926.

[3] *Ekman*, 1929.

[4] *Detwiler* (1929b) observed self-differentiation of transplants of the prospective limb district taken from a neurula, while *Suzuki* (1928) observed defects and even suppression of limb development after localized injury to the prospective limb material as early as at the beginning of gastrulation.

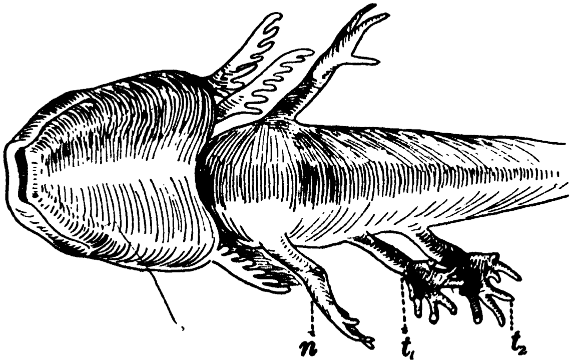

FIG. 68. Twinning of limb in *Amblystoma.* (From *Swett,* 1928) A left limb bud was split and a strip of strange tissue interposed to prevent reunion of the halves. The divided complex was then transplanted in dorso-dorsal orientation to the right flank, six segments behind the normal right fore limb (*n*). Result: Each half of the split limb bud has produced a separate limb (t_1 and t_2), and each unit has in itself become partly reduplicated. × 7.

The specific pattern of limb development is contained within the *mesodermal* portion of the limb bud, while the ectodermal covering of the bud behaves in a rather passive manner and yields to the growth pattern of the underlying mass. After transplanting the mesodermal component alone, one therefore obtains self-differentiation of a limb of the same perfection as if the ectodermal component had also been taken (Fig. 92).[1] Now, after splitting the mesodermal limb bud, *a whole limb may be obtained from each half* (Fig. 68). In fact, this twinning often occurs spontaneously in limb buds transplanted to heterotopic positions.[1] In turn, if an extra limb bud is superimposed by transplantation upon a normal limb rudiment, the merged materials produce a single uniform limb.[2] These remarks hold primarily for the free portion of the extremity, whereas the determination of the pectoral and pelvic girdles follows a slightly different course: the girdles are in a mosaic condition at a time when the finer details within the free limb are still undetermined.[3]

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[1] *Harrison,* 1918, 1931. The most convincing demonstration of the de-

Equipotentiality within organ rudiments in general.

It seems needless to point out that the behavior of the limb field district, as revealed in these experiments, conforms fully with that of the eye and the heart described before. Moreover, similar experiments were conducted with a number of other organs, such as nose, balancer,[4] ear,[5] gills,[6] tail,[7] liver,[8] with essentially the same results. Summarized these are as follows:

In a pre-gastrulation germ none of the prospective primordia possess definite self-differentiating tendencies; that is, most areas of the young germs are blank with regard to their future fate. Then comes a stage during which different areas of the germ draw specific and definitive assignments for a circumscribed developmental task, such as eye, tail, ear, limb, heart, etc. (see p. 320). This change does not, however, at the same time convey detailed instructions to

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cisive role of the mesoderm in limb development has been furnished by experiments in which limb ectoderm of one species was combined with limb mesoderm of another species: the composite limb took after the character of the species which had contributed the mesoderm (see Fig. 92); *Rotmann*, 1931, 1933. Yet, the presence of an ectodermal coat is necessary to permit the limb to attain its fully differentiated condition (*Balinsky*, 1935).

[1] *Swett*, 1926, 1928b.

[2] *Harrison*, 1918.

[3] *Detwiler*, 1918; *W. Brandt*, 1927; *Meyer*, 1926; *Swett*, 1928a.

[4] *Harrison*, 1925.

[5] *Kaan*, 1926.

[6] *Ekman*, 1922; *Rotmann*, 1931.

[7] *Bijtel*, 1931.

[8] *Holtfreter*, 1925.

the individual cells that are present, nor does it in advance commit the progeny of these cells to any final performance. *The dealing out of the final assignments to the elementary cells is an internal affair of the local field* within whose range they happen to lie. The dual character of the organ rudiment becomes thus very clear; constituting a field district, its cells are *collectively* engaged in specific actions; as *individuals*, however, they submit to the effects of these actions.

Fields acting beyond their original districts.

11. Certain fields, in addition to controlling the cells of their own district, *extend their domination over strange materials* which have come within their reach. Concurrently with the emancipation of local fields, the materials of the germ suffer a considerable amount of shifting (see p. 68) through which formerly distant regions are brought in close apposition. The results of such encounters depend on the degree of organizing activities prevailing in the parts coming in contact. If both are fully occupied with field activities of their own, their meeting may be of no consequence. If, however, at the time of the encounter, one of them is idling, as it were, and exhibits no specific formative activity, three things can happen: the inactive part may be modified by the active neighbor, or it may be left unaffected, or it may be incited to start some activity of its own. The ectoderm of the later germ furnishes examples of all three occurrences.

An illustration of the first occurs in limb development. When mesoderm of the limb bud is transplanted heterotopically, it grows out to form a limb. In doing so, it pushes the coat of strange epidermis under which it had been grafted ahead and impresses its shape upon the skin coat. In the whole process the ectoderm behaves as a *passive plastic membrane* of no formative tendency of its own.

As for the second case, we can point to the formation of the heart and pericardium which move into the ventral midline of the body (Fig. 66). Although they come to lie directly under the epidermis, the latter is not noticeably affected. In marked contrast to these two instances, however, ectodermal epidermis shows a very active behavior when it is approached by a differentiating *eye vesicle.* Then, as a result of the contact, it often produces a *lens.*

The origin of the lens.

The morphological development of the amphibian lens is as follows (Fig. 11, p. 65): as the lateral bulge of the eye vesicle approaches the skin and transforms into the eye cup, the skin within the area of contact thickens and forms a cushion-shaped body, the *lens placode.* As the eye cup deepens, the basal layer of the placode becomes curved with the convexity facing the cup. The curvature increases, until the plate is completely bent into a vesicle, the *lens vesicle.* This vesicle is then pinched off from the ectodermal covering and sinks into the hollow of the eye cup. The cells facing outward become a flat epithelium (*lens epithelium*), while the cells facing the eye differentiate into long and regularly arranged structures, the so-called *lens fibers.*

The lens is, therefore, by origin wholly extrinsic to the eye, but develops from the beginning in intimate association with it. This fact in itself does not yet prove the existence of a causal connection (cf. example 2), but experiments have clearly demonstrated that there is such a connection.

Example 56.[1]

A lens which depends upon the eye.

When the prospective eye primordium of one side is radically removed from the neurula or eye-vesicle stage of a grass frog (*Rana fusca*), the operated side remains permanently *eyeless* (see p. 256). But in addition, *the lens also fails to develop.* Since the lens-forming ectoderm was not injured in the operation, its failure to produce a lens must be ascribed to the elimination of some factor, specific or unspecific, contained in the eye vesicle and indispensable for lens development. That the factor is *specific*, is demonstrated by the reciprocal experiment, in which the extirpated eye rudiment of the grass frog is brought in contact with a strange skin region in which lenses do not normally arise. This can be done either by transplanting an eye vesicle under the skin of the belly, or by grafting a flap of belly ectoderm in the place of the presumptive lens ectoderm over the intact eye vesicle. In either case, *the strange ectoderm in contact with the eye vesicle readily furnishes a lens* the size of which matches the size of the eye bulb (cf. Fig. 95). This result is only obtained if the concavity of the cup, i.e., the retinal layer, is exposed to the skin; the tapetal layer is ineffective. Furthermore, the contact must be direct; an interposed layer of mesenchyme, however thin, prevents the effect. Embryos of newts, toads, and a few other forms have given the same results as the grass frog.

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[1] *Spemann*, 1901, 1912; *Lewis*, 1904, 1907. For a comprehensive review of the problem, see *Mangold*, 1931.

We learn, thus, that *the lens in these forms owes its origin to some specific action exerted by the retinal layer of the forming eye.* Evidently, the eye field, besides producing the retinal organization, exerts specific effects upon cellular masses lying outside the original field district. Specific organizing actions of this kind extending from one part of the body into an adjacent part have been called *inductions* (see p. 273). The eye "*induces*" the formation of a lens. But while this holds true for the grass frog and newt, other forms do not seem to fall in line.

Example 57.

A lens which develops independently.

The same experiment as in example 56, when repeated with embryos of the *water frog* (*Rana esculenta*), or of the *axolotl*, yields entirely different results. In these forms, the mechanical *removal of the eye-forming material*, or the suppression of eye development by chemical means, *does not preclude the formation of a lens* in the otherwise eyeless area (Fig. 69).[1] Although such independently differentiated lenses frequently fail to reach great perfection, the fact remains that in some instances *fairly ad-*

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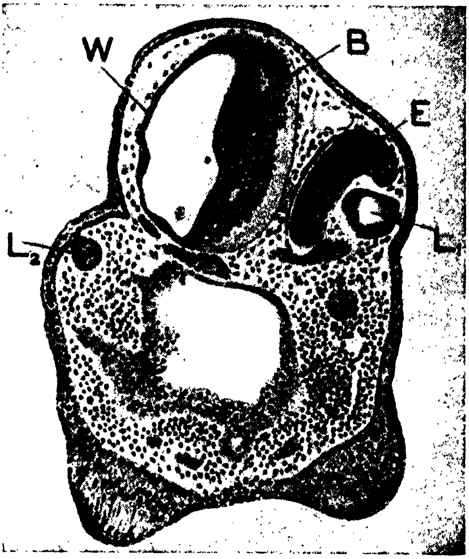
[1] *Spemann*, 1912.

FIG. 69. Differentiation of lens from lens-forming skin area in the absence of the eye in the water frog, *Rana esculenta*. (From *Spemann*) Despite early excision of the left brain half including the eye rudiment, a left lens (L_2) has been produced in its normal place. It is, however, retarded and undersized in comparison to the lens (L_1) of the undisturbed right side.

B, Intact right half of brain. W, Wound epithelium which has covered the defect of the left side. E, Right eye.



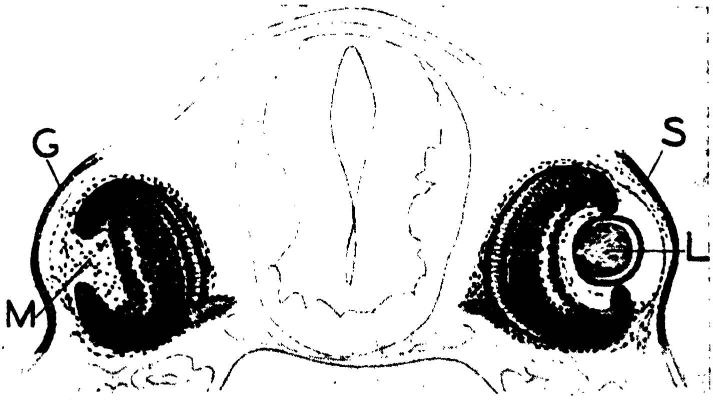


FIG. 70. Inability of body skin to form lens in the water frog, *Rana esculenta*. (From *Spemann*) A flap of belly ectoderm (G) grafted over the eye has failed to produce a lens. The eye of the opposite side has a lens (L) which has developed from the normal lens-forming ectoderm of that side. M, Mesenchyme in place of the missing lens.

vanced stages of lens differentiation can be obtained in the complete absence of an eye. The reciprocal operation, namely, the bringing together through transplantation of an eye vesicle with ectoderm other than that of the presumptive lens-forming area, seems to confirm the lack of determinative relationships between eye and lens; for, unlike the situation in the grass frog, *no lenses arise in strange ectoderm of the water frog* (Fig. 70).

This failure of the eye to provoke lens formation must be ascribed either to lack of inductive activities on the part of the eye or to lack of proper responsiveness in the skin. In order to decide between these alternatives, one had to expose an eye vesicle whose inductive powers were in doubt to ectoderm of known responsiveness. According to example 56, ectoderm of the toad embryo has this reactive capacity throughout; and in fact, when ectoderm was taken from the belly of a *toad* embryo and grafted over the eyeball of a *grass frog* embryo, it *produced a lens*, under the same circumstances in which the grass frog's own belly ectoderm has consistently failed.[1]

Reconciling the results.

This result demonstrates that even in forms in which the lens can develop independently, lens induction is among the capacities of the eye field. Continued experimentation has shown that the results described in examples 56 and 57 which at first appeared to be in sharp contrast, are merely the extremes of a line of continuous intergradations. In *Rana fusca* no lens is formed in the absence of the eye; in *Rana esculenta* or in *Amblystoma* a fairly complete lens can be formed under the same conditions. *Bombina*, however, represents an intermediate form[2] in that the development of a lens is initiated but remains rather rudimentary (a so-called lentoid). In *Rana fusca* practically the whole body ectoderm can respond to lens induction, while in *Rana esculenta* and *Amblystoma* the ectoderm with the exception of the normal lens-forming portion has lost this capacity. Again, however, *Bombina* assumes an intermediate position in that lens can be formed from any portion of the head ectoderm; that is, responsiveness to the lens-provoking agent is neither ubiquitous nor strictly limited to the lens-forming area.

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[1] *Filatow*, 1925.

[2] *Spemann*, 1912.

Even the most compliant ectoderm of the grass frog does not retain its responsiveness to lens induction permanently. Epidermis of an older grass frog larva is just as refractory as is the ectoderm of the younger water frog embryo. According to the principle of exclusivity (p. 99), the mere fact that the ectoderm sooner or later becomes engaged in actual histological differentiation necessitates the subsidence of responsivity to other stimuli. If we assume that this crucial step occurs later in the grass frog than it does in the water frog, we have accounted for the different responsiveness of the ectoderm of both forms to lens induction. This does not yet explain, however, why lens formation in the water frog is wholly independent of the eye, and why there is this general correlation between independent lens development and precocious loss of reactivity in the rest of the ectoderm. No satisfactory solution of this problem has as yet been advanced. But it is reassuring to know that these experiments, although they contain a renewed warning against unlimited extension of results from one form to another (see also example 3, p. 137), at any rate, disclose that the difference in the mode of lens determination among different amphibians is not of a fundamental nature; rather is it a matter of varying *degrees* of dependence and different rates with which responsiveness of the ectoderm subsides. So far as the lens-inducing capacity of the eye vesicle is concerned, all vertebrates thus far examined, including teleosts and birds, behave essentially alike.[1]

Induction.

While *induction*, as exemplified by the action of the eye upon the skin, seems to be a major instrument of development, it would be erroneous to consider it as *the* fundamental principle. At best, it is on an equal footing with the processes of internal organization and segregation taking place *within* a given field district which we have called *emancipation.* One is reminded of a similar duality in the evolution of a state or a nation which progresses as much by internal organization and differentiation — comparable to field emancipation — as by conquest and assimilation of neighbors — comparable to induction.

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[1] For full information, see *Mangold*, 1931b.

Lest the term *induction* lose all of its significance, it seems advisable to confine it to the *specific reactions* evoked by one organic part in another. When a person in a dense crowd is pushed around, one would hardly say that he has been "induced" to move in this or that direction. Similarly, if a surface layer of cells, such as the skin over a limb bud, is stretched out by the local growth of the underlying mesoderm (see above), its behavior is not essentially unlike that of a rubber balloon distending under inflation. Or, when one tissue approaches another tissue and dissolves it by enzymatic action, so that a perforation appears, as is presumably the case in the formation of the mouth or the anus or the gill slits in vertebrates, one is hardly justified in calling this an induction; the formation of a hole has been *produced,* but not *induced.* Intestinal secretions do not induce food to become digested; they simply digest it. However, in addition to, and partly superimposed upon, passive alterations of this sort, which are too obvious to deserve further comment, we observe true *inductions* whose criterion is the *specificity of the reaction of the affected parts.* A few additional examples may illustrate this type.

Example 58.

The induction of cornea differentiation.

The skin area which covers the eye after the lens has been pinched off undergoes a peculiar histological differentiation. It fails to develop glands, its pigments are dissolved, its cells become transparent, and between it and the front of the eye bulb a laminated stroma of mesenchyme appears (Fig. 11). Thus the transparent *cornea* of the eye arises. However, this occurs only *in the presence of an eye.* After removal of the embryonic eye rudiment ordinary opaque skin forms over the eyeless area.[1] Conversely, the heterotopic transplantation of an eye bulb under body skin [2] or the transplantation of a patch of strange skin over an embryonic eyeball [3] are followed by clearing of the cells, reduction in thickness, disappearance of glands; in other words, by the appearance of the typical characters of a cornea. Not only the whole eyeball, but also fragments thereof, as well as the lens alone, can produce this effect.

The reaction of the skin in these cases is quite specific and differs distinctly from the passive thinning out of skin under the influence of mechanical pressure.[4] It may, therefore, rightly be classed as a phenomenon of induction.

Example 59.

The induction of ear capsule formation.

The vertebrate labyrinth, a membranous sac differentiating from the invaginated ear vesicle (see p. 320), becomes early enveloped by a coat of mesenchyme which subsequently transforms into a cartilaginous capsule. It has been found that *after suppression of ear formation* by radical extirpation of the ear field district, *the skeletal capsule fails to develop.*[5] The materials normally building the capsule are derived from head mesenchyme, none of which is removed when an ear vesicle is cleanly excised. Therefore, it is not lack of proper building material that has caused the absence of a capsule, but the elimination of some condition essential for its production. Heterotopic transplantations, moreover, have revealed that the ear vesicle serves as a specific inductor rather than merely as a mechanical mold. *Mesenchyme surrounding such a grafted ear vesicle can form a typical ear capsule.*[6] One might contend that perhaps mesen-

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[1] *Lewis*, 1905; *Spemann*, 1912.

[2] *Wachs*, 1914; *Fischel*, 1917.

[3] *Groll*, 1923.

[4] *Cole*, 1922.

[5] *Filatow*, 1916.

[6] *Sternberg*, 1924; *Balinsky*, 1925; *Filatow*, 1927.

> chyme cells accumulate on the surface of any object and subsequently chondrify. This is contradicted, however, by the fact that *only certain types of mesenchyme* respond adequately with the formation of an ear capsule, while others either fail to react altogether, or accumulate, but give rise to different types of cartilaginous structures.[1]

A specific relation between ear vesicle and the reacting mesenchyme seems, thus, clearly indicated.

Utility of inductive relations.

This list of examples could be extended without difficulty but would show little else than that induction is a fairly common occurrence in development, especially in the formation of composite organs which receive their contributions from originally independent and spatially separated sources. Specific interactions of inductive character among the diverse constituents of an organ are almost a logical postulate. Unless such interactions existed, it would be incomprehensible that the complex structure of an organ could ever be accomplished in an orderly manner, in view of the considerable variations existing among individuals. The point is clearly illustrated by the development of the eye. It is obvious that with lens development being localized under the direct influence of the eye, and cornea development being localized under the direct influence of lens or eye or both, there is much greater assurance of having lens and cornea fitted into their correct places, than there would be if each part were formed in complete independence of the others, with no direct interactions controlling their combination.

The biological utility of the inductive principle is, therefore, unmistakable. Nevertheless, it is worth keeping in mind that every match of developing parts cannot be ascribed to induction. Numerous composite structures are known to arise in the embryo, whose components roughly fit into one another as if by coincidence, that is, without there being demonstrable, direct, inductive interrelationships. The two portions of a joint furnish a pertinent example.

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[1] Only axial mesenchyme produces an ear capsule while lateral plate mesenchyme differentiates supernumerary girdle fragments (see p. 367); *Balinsky*, 1925.

Example 60.

Parts that fit without inductive interrelation.

A shoulder joint consists essentially of a socket in the shoulder girdle into which fits the convex head of the humerus. The joint is to act as a ball bearing, and its typical shape is as essential for the mobility of the arm as the lens is for vision. Thus the assumption that one of the two matched parts of the joint may be formed in direct dependence upon the other does not seem far-fetched. However, when the rudiment of the free portion of a fore limb is removed in early stages (in anurans), it is found that *the shoulder girdle develops a typical socket,* as usual,[1] even though there is now no humerus present that could have determined its shape. Conversely, *the humerus of the isolated limb bud develops a typical convex head* in the absence of any shoulder socket (compare also Fig. 48).

Here we have then a case in which the two fitting portions of a composite organ, the joint, originate and assume their characteristic form independently. Although there can be no doubt that later mutual adjustments of detail take place between the two partners, comparable to the final grinding, polishing, and assembling of pre-fabricated parts in the building of a machine, the essential fact remains that the main outlines of the two component processes were established by independent activities. Examples of this type could also be listed in numbers; they prove that the mere association between two parts for a common function does not in itself justify inferences regarding the inductive dependence of one upon the other (compare also example 2).

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[1] *Braus,* 1909.

General remarks about induction.

Induction, like all field activity, is stereotyped and "blind." It produces its single-tracked results irrespective of whether this benefits the body as a whole or not; under normal conditions, it does, and under abnormal conditions, it often does not (see p. 275). A lens on the belly (example 56) is useless because the eye to which it belongs is of no service to the body, anyhow.

If induction is recognized as a fairly common *principle* of development, this should not be understood to mean that the *mechanisms* and agents through which inductive effects are produced are all of one type. As a matter of fact, an unbiased survey of the known instances of induction reveals such a variety of factors involved that it appears very doubtful that they should have a single common denominator.

Asynchronism of determinations.

12. The very fact that inductions occur proves that all parts of the developing organism do not attain the same degree of determination simultaneously. Some must be further advanced than others. Determination is measured by self-differentiating capacity. Self-differentiation, however, means inability to give up the initiated developmental course for some other course. This would preclude response to inductive influences. If, therefore, a self-differentiating transplant, e.g., an eye, can subdue surrounding parts, e.g., overlying ectoderm, and cause therein the formation of an organ, e.g., lens, which otherwise would not have been formed, this is evidence of a decidedly low degree of determination in the affected material. Thus, *the succession of steps in a chain of inductive processes reveals the order in which the determination of adjacent parts lags.*[1] The cornea lags behind the lens, and the lens behind the eye. The eye itself, however, has derived its self-differentiating and organizing capacity by emancipation from the medullary field.

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[1] *Spemann*, 1938, has called attention to the existence of a sequence or chain of inductive events of different order in the development of the eye.

The origin of the medullary field.

This takes us back to the *medullary field;* what do we know about its origin? Is it a primordial institution of the germ or the result of an autonomous emancipation? So far as one can tell from the existing evidence, in amphibians it is neither one nor the other; it is generated through *the inductive activity of the subjacent ento-mesodermal field,* the most extensive and consequential induction that has thus far come to our knowledge.

The Chorda-Mesoderm Field

Before reporting the pertinent experiments, we must digress to give a brief account of the shifts of the germinal areas of an amphibian blastula during the process of gastrulation.

Amphibian gastrulation.

The three germ layers of which the germ consists at the end of gastrulation are derived from distinct sectors of the blastula (Fig. 60, p. 305): (a) the bulk of the animal hemisphere represents presumptive *ectoderm,* i.e., presumptive epidermis and presumptive medullary plate; (b) the presumptive *entoderm* comprises the lower portion of the vegetative half of the blastula; and (c) the presumptive *mesoderm* lies in a band separating (a) from (b) in the near-equatorial part of the vegetative hemisphere. This ring of presumptive mesoderm is widest on the presumptive dorsal side of the germ and narrowest on the opposite side. We may call it the *marginal belt.* It contains essentially material of the similarly shaped gray crescent of the undivided egg (see p. 229). Its upper border divides the ectodermal materials destined to remain in the surface of the germ from the meso- and entodermal materials which will be invaginated into the interior. It is homologous, therefore, to the rim of the blastopore in the sea urchin (Fig. 43). If gastrulation followed the method of the sea urchin, the vegetative part of the germ would be transported into the interior simply by rolling in around this fixed line. In a remote sense, this is true. But the process is more complicated in amphibians; the main difference is that the rolling in does not take place simultaneously around the entire circumference but starts from a circumscribed area and only gradually spreads to the rest. The starting point is a groove which forms in the vegetative hemisphere near the widest portion of the marginal belt. The groove marks the appearance of the *blastopore.* Around it the surface materials begin to be rolled under, with the wide part of the marginal belt coming first. The border at which the superficial cell layer bends back on itself and disappears under the surface first is the so-called *dorsal lip of the blastopore.* As time goes on, more and more lateral regions are drawn into the process causing the lip to extend laterally in the shape of a horseshoe until it has finally completely surrounded the vegetative segment (yolk-plug) in the form of a circle. As more material sinks into the interior, the circle closes to a slit, much as one closes a purse. It must be understood that while the geometric form of the blastopore undergoes this slow transformation, the cellular materials composing its outline change continuously, comparable to a conveying belt which moves in one direction on the surface and in the opposite direction underground while the turning points remain fixed. In this process of invagination the wide part of the marginal belt which marks the later back of the embryo not only gets a head start but also proceeds much faster into the interior than does the rest of the surface. Under considerable stretching out it applies itself closely to the overlying ectoderm so as to form an inner lining, or substratum, for the latter. Within an area roughly coextensive with the substratum, the ectoderm then begins to transform into *medullary plate* (see Fig. 109). The substratum itself produces the *notochordal plate,* flanked by *mesoderm.*[1]

Experiments have shown that the medullary plate forms not only in contiguity with, but in direct causal dependence upon, the underlying mesodermal material. The crucial evidence has come from transplantation experiments in which part of the mesodermal substratum was made to face ectoderm of other than presumptive medullary destination.

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[1] For further details, see, for instance, *McEwen,* 1931.

Example 61.

Self-differentiation of chorda-mesoderm grafts.

Presumptive *chorda-mesoderm* can be obtained from a medium-aged gastrula by cutting it out at the dorsal lip of the blastopore around which it is being invaginated. Such a fragment, when grafted into the flank or belly region of another germ of the same or slightly younger age, persists in its invagination movement and disappears from the surface by rolling in under the ectoderm of the region of implantation. Another method consists of inserting the fragment into the blastocœle where it settles in some random site and in the course of gastrulation is pressed against the overlying ectoderm. Subsequently, two things happen which should not be confused.

Firstly, the grafted fragment exhibits remarkable *self-differentiating power* in that it gives rise to a more or less complete mesodermal axis of an embryo;[1] it transforms into straight notochord flanked on either side by a row of typically segmented myotomes, pronephros, and lateral unsegmented mesoderm.

In this respect the transplant behaves essentially as an explanted or transplanted primordium of an eye, ear, limb, or heart would behave in similar circumstances (see p. 312). And just as irregularly excised organ rudiments nevertheless give rise to complete and harmonious organs, thus proving their internal equipotentiality (p. 328), so the transplanted chorda-mesoderm district usually assumes bilateral symmetry even if taken from an asymmetrical, somewhat lateral position.[2] Apparently, by the time of transplantation this area behaves still as a unit, and no particular subcenters for the formation of notochord, somites, pronephros and mesoderm have yet been established. In line with what we have learned about other organ rudiments, the integrity of the field carried by the graft has enabled the subordinated mesodermal structures to assume a *whole* chorda-mesoderm pattern instead of merely that portion of it which the piece would have formed in its old place. This chorda-mesoderm field, furthermore, can engage surrounding stray mesoderm of the host embryo and utilize it together with its own cells. If donor and host belong to different species, *chimaeric* organs result which are uniform in outline and build but are mottled, with patches of host cells interspersed with groups of transplant cells.[1] This harmonious molding of uniform organs from a mixed cell material reminds one of the carving of a statue from a conglomerate rock. So far, the behavior of the transplant conforms with that observed in other organ rudiments. The only difference lies in its precocity, for it will have been noted that no other parts of the germ tested by explantation or transplantation have exhibited self-differentiating capacity as early as at the stage of gastrulation (examples 48, 51). Aside from this time difference, the situation is fundamentally the same. The *transplant continues its development by virtue of its own field.* So much for the first point — the self-differentiation of the transplant.

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[1] First observed by *Lewis*, 1907.

[2] *Bautzmann*, 1926; *Ekman*, 1936.

Inductive action of the chorda-mesoderm graft.

Secondly, however, the new mesodermal axis *induces the overlying ectoderm to give rise to a medullary plate* and, in further consequence, *a whole medullary system* of such orientation, proportions, arrangement and size as to match the underlying mesodermal structures.[2] The composite product, consisting partly of transplanted strange material, partly of induced local material, represents an essentially typical *secondary embryo* (Fig. 71). Just as the eye vesicle, in addition to elaborating the organization of its own materials, induces the formation of a lens in the overlying skin, so the mesodermal axis, in addition to continuing with its own segregation and differentiation, induces the formation of the medullary plate in the strange ectoderm with which it has been forced to make contact. For direct contact is necessary in order to obtain the inductive effect.

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[1] *Spemann and H. Mangold,* 1924.

[2] This phenomenon which was first observed by *Spemann and H. Mangold* (1924; also brief announcement in *Spemann,* 1921) has been reported repeatedly since; it is usually referred to as "induction of a secondary embryo" which seems quite proper so long as one remains aware of the complexity of the processes involved.

The impressive demonstration of this conspicuous induction effect, revealing the specific dependence of the development of such an important organ as the central nervous system upon some other organ, undeniably has been one of the major achievements of modern embryology. The prominence of the effect, together with the fact mentioned before that the chorda-mesodermal substratum is decidedly *precocious* in its possession of self-differentiating and inductive powers, accounts for the names of "*organizer*" or "*organizator*" or "*organization center*" under which the chorda-mesodermal field district is frequently referred to.

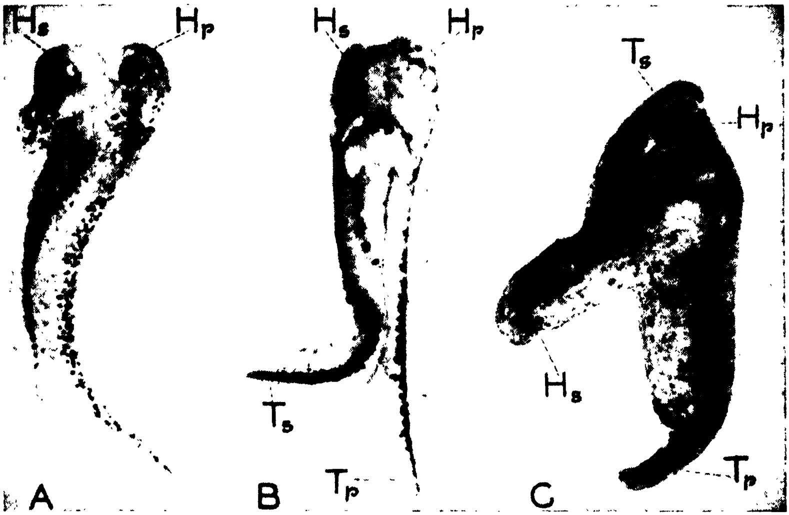

Fig. 71. Production of secondary embryos by implantation of fragments of presumptive chorda-mesoderm. (From *Holtfreter*, 1933)

A, Secondary head on ventral side of primary embryo.

B, Almost complete secondary embryo on ventral side of host.

C, Complete secondary embryo.

In A and B there is strict correspondence between orientation and regional organization of the primary and secondary embryos, while in C there is no correspondence whatsoever. For an explanation of the two types of results, see page 372.

H_p, Head of host embryo. H_s, Head of secondary embryo. T_p, Tail of host embryo. T_s, Tail of secondary embryo. × 12.

Is the "organizer" an organizer?

These terms, tentatively introduced by *Spemann* to designate the situation reported in the preceding paragraphs, while inoffensive when applied in this restricted sense, have often been mistaken as to their implications. The term "organizer" intimates that there is concentrated in a given location of the germ a *monopolistic* agent from which all subsequent organization of the germ would be derived. Whether or not the original experiments were conducive to such a monopolistic concept, it has certainly not found support in the numerous studies that have since been devoted to these problems. Although nobody in possession of the facts would probably think of taking the term literally and regard the "organizer" as really *the* organizer of the germ, there is the danger that the far greater number of those less familiar with the situation might succumb to the suggestive terminology. For this reason, it seems preferable to desist from using the ambiguous term altogether. Out of sheer respect for the admirable work which led to its creation, one might be reluctant to drop the term "organizer," were it not for the fact that recently *Spemann* himself has taken a very critical attitude toward it and all but cautioned against its use.[1] Therefore, in the following discussion the non-committal term "*chorda-mesodermal field district*" will be used instead.

Another point needs some brief comment. The inductive phenomena described in example 61 are frequently described as emanating from the *dorsal blastoporal lip;* this is not quite accurate. The dorsal blastoporal lip is not a definite and stable body of cells but merely a geometrical mark whose material composition is subjected to continual change; we have compared it with the turning point of a conveying belt (p. 342). Not even for a short while does it retain its material identity. Only in connection with an exact statement as to how far gastrulation has proceeded, can one refer to the dorsal lip in any material sense, because only so is it possible to state just what level of the invaginating chorda-mesoderm is then rounding the corner.

One would have been justified in retaining the term "organizer" in its full meaning if the progressive organization of a germ had been proved to be but a chain of successive inductions starting from this center and spreading over the rest of the germ like a blaze. Since we have seen, however, that the very development of the "organizer" itself is principally its own internal affair involving autonomous emancipation of partial districts, such as chorda, musculature, pronephros, inside the common cell mass, we realize that the problem of organization is actually contained in a nutshell in what happens inside of the "organizer" itself, regardless of what it does to other parts. Hence, to assume that the problem of organization could be fundamentally solved by exploring merely the inductive actions of the "organizer" upon surrounding parts of the germ, seems like looking for the fire outside of the house while the blaze is within. In our present rating, the induction of a medullary system by the chorda-mesoderm field is neither the igniting spark of development nor an act of unique significance for the understanding of the general problem of organization, but simply an important step in development. As such we must give it some further consideration, particularly as it is the best investigated case of induction.

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[1] *Spemann*, 1938, page 367.

Twinning the chorda-mesoderm district.

The fact that the chorda-mesoderm district represents a harmonious-equipotential partial system throughout early development, accounts for two experimental results discussed on an earlier occasion. It was reported in example 35 that the permanent inversion of a frog's egg in the two-cell stage often leads to the formation of twin embryos; for this we can now offer an explanation. When the egg is turned upside down, the heavy yolk flows down in both blastomeres, mostly along the cleavage plane; as a result there appear two separate portions of vegetative material instead of one contiguous mass as usual. Since invagination always starts in the vegetative mass near the marginal belt (see p. 342), the subdivision of the vegetative mass into two discrete portions creates *two centers of invagination* in the inverted egg. Consequently, the presumptive chorda-mesoderm district is split; part of it is rolled in around one blastopore, and the rest around the other. However, just as a lateral fragment of the chorda-mesoderm district develops into a whole axis system when excised and transplanted (p. 343), so in the present case also *both halves give rise to bilaterally symmetrical axial systems and,* moreover, *induce bilaterally symmetrical medullary systems* in the covering ectoderm.[1] The result is a double embryo. This is merely another instance of the twinning of split organ primordia divided prior to the moment of further inner emancipation (p. 329).

The role of the chorda-mesoderm district in fused germs.

A case comparable to the formation of a single organ from two fused organ primordia has been reported in example 47.

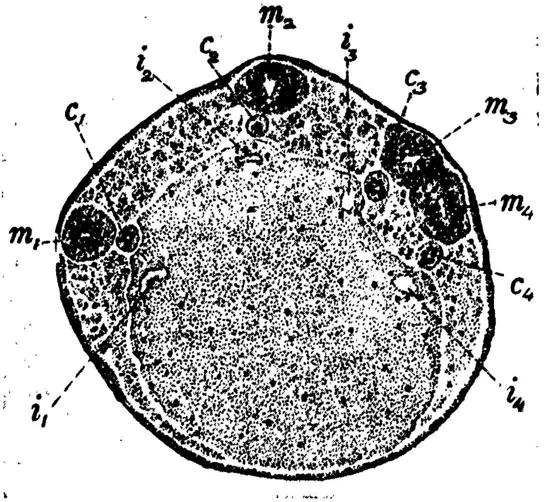

FIG. 72. Formation of quadruplet embryo from two newt eggs fused crosswise in the 2-cell stage as in Fig. 58. (From *Mangold and Seidel,* 1927) Cross section. m_1, m_2, m_3, m_4, Neural tubes. c_1, c_2, c_3, c_4, Notochords. i_1, i_2, i_3, i_4, Lumina of primitive guts. Common yolk mass. × 42.

There it was described that when two amphibian germs of the two-cell stage are joined crosswise, single uniform embryos can arise (Figs. 58, 59). But this is by no means always the case. Many of these germs have developed more than one, and up to four separate embryonic systems (Fig. 72).[1] A plausible explanation for the variability of the results has been offered on the basis of two assumptions; first, that the chorda-mesodermal field district is already present, although not necessarily active, in the *undivided* egg and assumes an eccentric position; second, that the first cleavage plane divides it more or less at random, so that in one group of eggs most of it will have become allotted to one of the first two blastomeres, while in another group both blastomeres

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[1] *Penners* and *Schleip,* 1928; *Wittmann,* 1929.

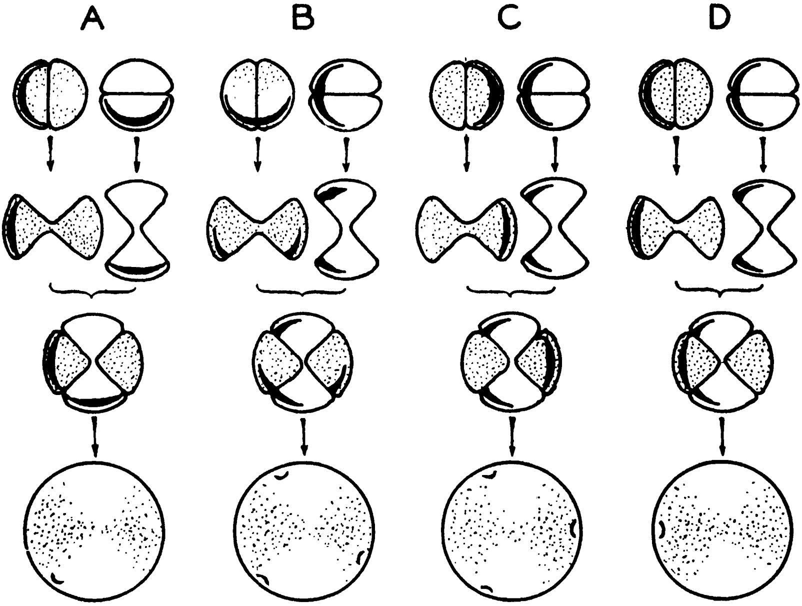

FIG. 73. Diagram illustrating the four principal combinations resulting from fusion of two eggs in the 2-cell stage (see Fig. 58). Cases in which first furrow lies either perpendicular or parallel to gray crescent (as the forerunner of the chorda-mesoderm district).

Top row: Eggs before fusion, showing position of gray crescent.

Second row: Eggs dilated preparatory to fusion.

Third row: Eggs after fusion.

Bottom row: Beginning gastrulae developed from fused eggs, showing position of blastopores (one for each contiguous gray crescent field). A and D furnish single giant embryos (Fig. 59), B and C furnish triplets. Twins and quadruplets (Fig. 72) result from intermediate combinations.

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[1] *Mangold and Seidel,* 1927.

will have received more nearly equivalent portions of it. Since, as is shown in Figure 58, the eggs are merged by putting one across the other, the two fractions of a split chorda-mesodermal field district are widely separated. Thus, a variety of combinations is obtained in accordance with the varying position of the first cleavage plane. After the fusion the four blastomeres may appear in any of the conditions illustrated in the diagram, Figure 73.[1] Fractions of the original field districts which come to lie side by side merge again, and in the later course of events *each continuous district produces one axial system and one embryo.* In combinations A and D the chorda-mesodermal districts of both eggs are joined into a common mass, thereby establishing a single embryo (Figs. 58C and 59). In the other combinations those islands of the original districts which have failed to join because of greater spatial separation establish axial development of their own, leading to double, triple, or quadruple formations (Fig. 72).

Prelocalization of the chorda-mesoderm field.

What interests us most in this interpretation is that it seems to confirm the first of the above two assumptions: a *definite eccentric localization of the chorda-mesoderm district in the egg as early as at the beginning of segmentation.* This is further corroborated by the following experiment.

Example 62.[2]

When a newt egg of the two-cell stage is transected by a ligature along the first cleavage furrow, both halves continue to segment. With regard to further development, however, the pairs fall into two groups. In one group complete and proportionate embryos developed from both halves (as in Fig. 34), whereas in the other group only *one half produced a typical*

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[1] The diagram considers only the extreme cases in which the first cleavage plane divides the field district nearly symmetrically or lies at right angles to this position. The results of intermediate positions can be easily extrapolated.

[2] *Ruud,* 1925.

embryo, while the other half remained a mass of unorganized tissue.

These results can only be explained on the assumption that the undivided egg already contains a forerunner of the chorda-mesodermal field district in a definite eccentric localization, and that the arbitrary position of the first cleavage plane results in some eggs having all or most of this district allotted to one blastomere, while the other blastomere receives little or none of it. The latter, therefore, can form no chorda-mesodermal axis and no medullary system and, hence, remains shapeless. But, if one restores to such an unorganized half a chorda-mesodermal field district by grafting into it some invaginating material of a normal germ, one can still turn its development into a typical course.[1]

Tracing the chorda-mesoderm field to the gray crescent.

There is good evidence, therefore, that the unsegmented amphibian germ is not strictly a harmonious-equipotential system, but has already undergone some crude inner segregation through which certain specific properties, as for instance, those responsible for the later chorda-mesodermal differentiation, have become concentrated in a localized portion of the egg.

In the blastula and during subsequent gastrulation the center of the chorda-mesodermal field district lies in the widest (dorsal) portion of the marginal belt (Fig. 60). The plane of symmetry of the *marginal belt*, however, *becomes the median plane of the embryo;* it is along this plane that the first gastrula invagination occurs. On the other hand, we remember that the median plane of the embryo also coincides with the plane of symmetry of the *gray crescent* of the undivided egg (p. 229). Thus, a definite relationship between gray crescent and marginal belt suggests itself, and when the materials of the marginal belt are projected upon the surface of the undivided egg, we find that they cover approximately the area of the gray crescent. The gray crescent, as one will remember, arises from a peculiar local modification of protoplasm in the cortex of the egg prior to, or after, fertilization, and we feel assured that its properties are in some way distinctive and specifically related to the establishment of the future chorda-mesodermal field.[1] It was with this fact in mind that we could state above (p. 231) that *the morphological construction of the egg cytoplasm anticipates in a certain measure the basic pattern of the embryo.*

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[1] *Bautzmann*, 1927.

Lack of medullary formation in absence of chorda-mesoderm.

The positive proof of inductive medullary formation under the influence of grafted chorda-mesoderm has been supplemented by experiments which show convincingly that the *normal* nervous system arises in the same way and that it fails to develop if contact between the gastrulating chorda-mesoderm and the ectoderm is prevented. When young germs are treated with dilute salt solutions, this disrupts gastrulation movements and causes evagination instead of invagination of the entoderm and mesoderm (Fig. 74). The latter continue to develop in self-differentation, while the empty bag of ectoderm, devoid of mesodermal substratum, remains in primitive condition without producing a medullary system.[2]

The post-gastrulation fate of the germinal fields.

Let us now return to the chorda-mesodermal field at gastrulation and follow it in the opposite direction, i.e., toward older stages. As we have already mentioned, it suffers an inner segregation into sub-fields, the principal segregates being a median notochordal field, and bilateral somitic, pronephric, and hypomeric fields. Each of these then takes charge of the development within its domain. The results soon become evident in the appearance of notochord, myotomes, pronephros, lateral plates, etc. Simul-

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[1] *Brachet*, 1917. Compare also the stimulating discussion of this matter by *Dalcq and Pasteels*, 1938.

[2] *Holtfreter*, 1933d.

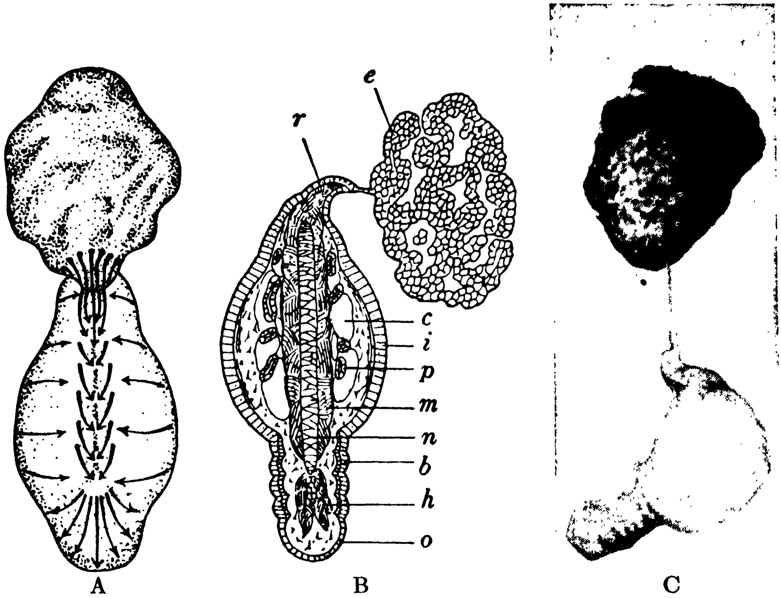

FIG. 74. Complete exogastrulation of an *Axolotl* germ. (From *Holtfreter*, 1933) Rearing the germ in dilute Ringer solution has had the effect of reversing the usual gastrulation invagination into evagination. Mesoderm and entoderm (lower mass) are thus turned inside out, but remain attached with their rear end (the blastoporal rim) to the empty sac of ectoderm (upper mass) into which they have failed to move. In the evaginated, ectoderm-free complex the mesodermal derivatives lie innermost, while the entoderm covers the surface. Note the high degree of organization in this fragment of embryo, in contrast to the lack of organization in the ectodermal part which notably failed to develop the nervous system. Note also the differentiation of contractile myotomes in complete absence of innervation (see p. 444).

A, Diagram indicating the gastrulation movements; stretching and dorsal convergence are prominent.

B, Diagrammatic longitudinal section through the later embryo.

C, Photograph of living germ at the age of 8 days.

e, Ectoderm. *r*, Extroverted rim of the blastopore. *c*, Coelomic cavity. *i*, Intestinal epithelium. *p*, Pronephric tubules. *m*, Myotomes. *n*, Notochord. *b*, Branchial pouches. *h*, Head muscles. *o*, Oral entoderm. × 9.2.

taneously, there occurs the induction of the medullary field. The latter, in turn, undergoes a parcellation into more restricted fields, such as those for eye and brain. The medullary field itself also exerts inductive effects. Among the first to occur is the induction of the *nasal field* in the anterior portion of the head, and that of *ear fields* at the level of the hindbrain. These inductions are essentially of the same order as the process of lens induction described before. Evidence for the existence of these local fields was reported in example 51. There it was also stated that a local field does not dissipate immediately after it has acted, but persists for some time. The same is true for *the chorda-mesodermal field* whose *inductive power survives the actual formation of the notochord and the myotomes*, as is shown by the following experiments.

Example 63.

Induction of medullary plate by late chorda-mesoderm.

> If, instead of transplanting the invaginating marginal material of the early gastrula, as in example 61, we transplant part of the *fully* invaginated mesodermal substratum of the finished gastrula under the ectoderm of a younger host, the transplant which had already once before induced a medullary plate in the donor germ, repeats this performance and once more *provokes the formation of a medullary plate* and all the dependent structures that go with it.[1] Even still later stages can be used. Fragments of notochord, taken from a donor which has long had its own medullary system, and transplanted into hosts of gastrulation age, also induce the formation of medullary systems in the host ectoderm [2] although the grafts themselves have become too rigid and firmly determined to form anything but what they already are, namely, chorda.

This same persistence of the field is observed again in the induced medullary organs. The inductive action of the chorda-mesodermal field endows the medullary system with self-differentiating capacity; that is, once the medullary system has been established, it can proceed with its typical differentiation even in the absence of further assistance from the outside.[1] As will be discussed on a later occasion (p. 494 f.) such independently developing nervous systems are not normal in every respect, but they can be recognized, at any rate, as decidedly nervous types of differentiation. In this state, the medullary system can still induce productions of its own kind in younger germs.

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[1] *Marx*, 1925.

[2] *Bautzmann*, 1928.

Example 64.[2]

Induction of medullary formations by medullary plate.

> When fragments of late medullary plates are transplanted under the ectoderm of a host which has not yet gastrulated, the transplants continue to develop brain parts in self-differentiation. But, in addition, they affect the host ectoderm so that simultaneously with the development of the host's own nervous system *supernumerary medullary inductions* appear in the vicinity of the graft.

Evidently a graft of pure medullary material carries a field which still has inductive powers. The fact that the affected host ectoderm produced supernumerary formations rather than joined the grafted medullary system is obviously due to the difference in age and maturity between graft and host cells. A similar situation was reported in example 51. In the present experiment the field district was grafted under still undetermined ectoderm. In example 51 young and undetermined ectoderm was grafted into the domain of otherwise undisturbed fields. The effects are essentially the same, and both experiments supplement each other.

Summary: Progressive organization of the amphibian egg.

Let us now summarize the conclusions arrived at thus far. The earliest field to be demonstrable in action is the chordamesodermal field. It gives rise by emancipation to fields of lower order ("sub-fields") within its own district. Concurrently, it establishes the medullary field by inductive action. Gradually local sub-field districts emerge, such as those of the eyes, lenses, ears, noses, gills, balancers, mouth, etc., derived partly from the medullary field, partly from the chorda-mesodermal field, and partly possibly under the control of both. These local fields subsequently enter into competition for the available material and outline their mutual realms in accordance with their relative strengths. The final outcome is a mosaic of localized field districts (e.g., organ rudiments) with a high degree of autonomy and self-differentiating power. This result is achieved in two different ways: firstly, by the emancipation of sub-field districts, more restricted in scope, within original, more comprehensive, field districts; and secondly, by the conferring of field characters upon outlying materials, such as indifferent ectoderm (so-called induction). *The local sub-fields are transitory stages in the transition of the germ from a state of indeterminacy of parts into a final stage where a maximum of definiteness has been acquired by each part* (see p. 74). *This transition is gradual and does not proceed at an equal pace in different parts.* This is essentially the answer to the questions of page 297.

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[1] *Mangold*, 1929c; *Holtfreter*, 1931b.

[2] *Mangold and Spemann*, 1927.

The Reactivity of Cells

The relation between field and elements.

Up to this phase, we have paid little attention to the problem of just how fields deal with the cells which have fallen under their domination. *Are the affected elements passive or active partners in the deal?* Are they passively forced into a definite course of development, or is it not rather that they are sensitive to directive stimuli and react by virtue of their own powers? Does the field *impart* upon subordinate parts distinctive properties, or does it merely select and *activate* appropriate reactions from among a store of circumscribed potencies preformed in each individual part? In short, if we consider the individual parts of a germ as the actors in a play, does the field teach them their roles, or does it merely furnish them cues as to which part of their stock repertoire they are to reproduce?

These questions have invited much speculation; It seems now that they cannot be answered by a clear cut decision in favor of one or the other alternative. The known facts suggest a compromise solution, namely, that the parts — cell groups and cells — behave in some regards passively and in other regards actively; some properties are imposed upon them from without as novel, while others have been their very own from the beginning.

Evidence of reactive behavior of the elements.

For an illustration of this statement let us turn back to example 48. It was described there that young ectoderm cells transplanted into the later brain region are drawn into the local course of development and utilized in the building up of a uniform chimaeric brain along with the native materials of the location. It must be stressed that the grafted cells have not by any means produced a separate brain, but have taken part harmoniously in the formation of the host brain which was in progress (Fig. 61C). This same fact — submission of materials of different origin under a common plan — has been repeatedly observed in experiments of this kind. (Compare also example 61 and Figure 17 — chimaeric cartilage.) Now, it is inconceivable that the irregularly outlined patches of grafted tissue should have developed in the fashion of inlaid work; that their cells should have craftily arranged themselves in such a shape as to fit exactly into the equally accidental and equally irregular gap in the host tissue. One could imagine that a cell group might have a certain capacity to build up a brain, an eye, an ear, all by its own; but it is beyond our imagination that cells should be able all by themselves to produce the infinite variety of morsels of brain, eye, or ear, which compose the chimaerical organs of the experimental germs. Clearly, then, in these *morphogenetic* processes masses of cells behave as passively as a mass of clay in the hands of a sculptor. Similarly, the outlining of the cylindrical notochord and the carving of the segmented somites from the bulk of dorsal mesoderm shows the cells at the receiving rather than at the producing end.

However, as has been pointed out already on an earlier occasion (p. 311), the cells of the graft in example 48, although they were molded into a common form together with the cells of the host, *grew according to their innate growth potential,* so that the *tempo* with which the developmental plan is put into effect appears as a constitutional property of the cells themselves. Cells taken from a different species than the one furnishing the field *obey the strange field adequately.* However, they do this *at the pace of their own species.*

Here we encounter the first demonstration of the fact that the same field influence can lead to somewhat divergent results when acting on material of different origin. To use an analogy, we may compare the situation to the formation of a whirl pool. A swift current erodes rock. Whirls arise where resistance changes the flow. Their shape, speed, and depth depend on the course of the river and the shape of the shore. Once a vortex is established, it begins to impose its shape upon the bed. Thinking of inductive processes, we might compare the swirling current to the action of a local field; just as the splitting of a field district yields two fields with separate centers, so the splitting of a whirl results in the appearance of two whirls separated by a quiet no-man's-land. The whirl begins to erode the rock; but in doing so, it encounters uneven resistance, due to the inequalities in the consistency and composition of the rock. The softer parts are washed off at a faster rate than are the harder parts; therefore, the same whirl will create a different pool, depending on whether it flows through soft clay or through hard granite. And if soft and hard strata alternate, the erosion pattern will become corrugated. Consequently, the eroded bed is not a true reproduction of the whirl, but a distorted image determined as much by the properties of the molded materials as by the molding action of the current.

Passive and reactive changes.

Translated into terms of embryonic development, this means: different protoplasms which differ in their constitutional properties, such as protein character, metabolic rate, viscosity, electric charge, surface tension, etc., necessarily react somewhat differently to the same field actions. One realizes, therefore, the limitations of field action: a field cannot force any material into any formation to which the material is not suited. This applies to the *passive* properties, such as pliability, cohesion, etc., as well as to the *reactive* properties of the cells. That is, *a field cannot make any cell produce any specific response unless that cell is intrinsically prepared to do so.*

If one turns back to the outline of development given in Part One, one will find that in some phases the individual cell element appears to play a relatively passive role compared with what happens to the tissue as a whole. This is particularly true in the case of morphogenetic movements, shifts, foldings, ruptures, and the like (see also above, p. 330). In producing this class of phenomena, field action is only restricted by the technological properties, the material constants, of the materials used in the construction. On the other hand, however, one will note many features in which the activity of the individual cell prevails, particularly cytological and histological differentiation proper, and in producing these phenomena, field action is bound to avail itself of that limited choice of specific innate potencies with which each cell is endowed. Thus, while in *morphogenetic* processes the *passive* aspect of the cell fate is in the foreground, in the *histogenetic* processes the stress lies on the *reactive* aspect.

Experimental information concerning these matters has come from two sources: first, the raising of isolated undifferentiated cell groups in indifferent surroundings, and second, the response of materials transferred from a germ of one species to that of another widely distant species.

Example 65.

Isolated cells manifest intrinsic differentiation potencies.

Numerous experiments have been executed in which fragments of early amphibian germs were reared in body cavities ("interplantation"; see p. 156) or in salt solutions ("explantation"; see p. 253). Since *body fluids* are usually contaminated with some chemical principles provoking certain cell reactions (see later, p. 384), they cannot be considered as strictly neutral media; but the environment provided by a *salt solution* is sufficiently indifferent to permit cells to manifest their intrinsic capacities in the purest possible manner. Under these conditions, even small fragments of a *blastula* continue to differentiate histologically, while their morphological development remains poor.[1] They are shapeless; their interior is rather chaotically arranged, but *the histological differentiation of their cells is often of an amazingly normal appearance.* Each cell type found in such explants can be definitely identified and resembles closely the corresponding histological elements of a normal embryo of comparable age of differentiation. One observes in the explants typical epidermis, nerve cells, glandular cells, muscle cells, chorda cells, cartilage cells, pronephric cells, etc., disposed in irregular masses. Mostly cells of identical character are joined together in small groups, but there is no special order in their arrangement. Ectodermal fragments differentiate epidermis, entodermal fragments produce various types of intestinal tissue, and fragments from the marginal belt give rise to a wide variety of mesodermal characters. When reared in body cavities, especially in the orbit of the eye, ectodermal cells transform frequently into chorda cells and muscle cells.[2] Special attention must be called to the fact that the cells tested in these experiments had been explanted at early stages (blastula, early gastrula), that is, at a time at which, as we have seen, their individual fate had not yet been fixed.

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[1] *Holtfreter*, 1929a; *Erdmann*, 1931.

[2] *Kusche*, 1929.

The cell repertoire.

For the moment, we shall not concern ourselves with the question as to why these fragments have entered one or the other direction of differentiation,[1] but center our interest on the fact that the histological differentiation of the explants has been so perfect and unequivocal, notwithstanding the lack of morphogenesis and organogenesis. Each cell turned out to be either a distinct muscle cell or a distinct chorda cell or a distinct nerve cell, etc., but there appeared neither novel types nor intermediates, no cells that would have a little of this and a little of that character. This *discreteness* of cell differentiation in vitro is in marked contrast to the practically unlimited range of variability, abnormality, and confusion of the external conditions under which these cells had been reared. This forces one to conclude that cells possess *an intrinsic latent repertoire of potencies* through which they are enabled to execute a limited number of discrete histological differentiations; a type of differentiation not provided for in this repertoire can never be effected. In other words, the complicated and minutely controlled procedure according to which an indifferent cell gradually transforms into either a muscle fiber or a nerve cell or a cartilage cell, etc., cannot have been imparted upon the cell by extrinsic action, as speech is taught to a child; because how could the positively unorganized conditions of a salt solution have procured the necessary highly specific instructions? It would be inarticulate noise rather than instructive speech that the cells would hear from the deranged environment. But inasmuch as they respond in clear cut terms, we realize that they could not possibly have depended upon external instruction for their vocabulary.

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[1] In some instances the differentiation of the explants or interplants proceeds roughly in the direction of the prospective fate of the area from which the cells are derived; this is especially true of explants from the precocious marginal zone. In other instances, however, cells have deviated considerably from their prospective course; for instance, when animal cells, representing presumptive ectoderm, differentiated into chorda (*Bautzmann*, 1929a).

Therefore, we repeat: *each cell inherits from the egg cell a limited assortment of discrete, definite, and clearly outlined potential mechanisms for the different lines of histological differentiation familiar to the species.* From this store of "differentiation potencies" (see p. 286) field influences activate electively a definite one for realization. We have been led, thus, by way of the isolation experiments, to postulate the same principle of "*discreteness*" of differentiation which we had originally derived from mere observation (p. 97). Whether the differentiation potencies of the cell are based on properties of the nucleus or of the cytoplasm, has not yet been ascertained.[1]

The genetic limitation of the cell repertoire.

The fact that each cell is bound to react exclusively in accordance with the standards of the species to which it belongs, has been referred to above (p. 100) as the principle of "*genetic limitation.*" By clever application of this principle, the most elegant experimental dissociation between the active and reactive components in the determination of embryonic characters was obtained.

Example 66.[2]

The head of the young larva of the *newt* carries ventrolaterally two rod-like excrescences, the so-called *balancers* (Fig. 77). The larva of the *axolotl*, on the other hand, lacks them. The problem was to find out whether the balancer field of the newt, if confronted with undetermined axolotl ectoderm would be able to make these latter cells of a balancerless species form a balancer. Thus, belly ectoderm from a young axolotl germ was grafted over the lateral head region of a newt larva prior to the time when the balancer was due to appear. *No balancer developed in the strange skin graft.* The axolotl cells apparently did not know how to respond to the strange newt demand. In the reciprocal experiment, however, *belly ectoderm of a newt* (the balancered species) *when grafted over the head region of the axolotl* (the normally balancerless form) *did develop a balancer.*

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[1] The observations on "somatic mutations" of cells indicate a nuclear foundation of differentiation potencies in that there is a striking correlation between chromosomal aberrations and the morphology of the mutant cells (*Stern*, 1936).

[2] *Mangold*, 1931a.

We are confronted with the surprising result that newt belly skin grafted to an axolotl head has, under the influence of the local field, complied with the *locality*, but has done so in the manner characteristic of the *donor* species and contrasting with that of the *host* species. The cells have reacted to the lateral head field of the *axolotl* to the best of their *newt* knowledge, according to which a balancer is the proper thing to form on the side of the head.

Xenoplastic grafts.

The following examples are possibly even more instructive:

Example 67.[1]

Anuran tadpoles possess no balancer but carry on the ventral side of the head, directly behind the mouth, two *suckers* — characteristic epithelial pads secreting a sticky substance. In urodele amphibians there is no trace of these. Now, if undetermined ectoderm from the trunk region of an *anuran* embryo is transplanted to the ventral side of the head of a *urodele* embryo, *the graft develops suckers in the typical anuran position behind the mouth.* For the urodele host this is, of course, a novel experience.

This experiment simply amplifies example 66. The anuran cells when subjected to the *post-oral field* of the urodele germ, behave as if they were in the post-oral field of an anuran and form anuran suckers. Evidently the cells are not being transformed directly through the local field factors, but merely receive their cues and then transform themselves accordingly. Similar results are obtained when the *oral* region of an anuran or a urodele germ is covered by body ectoderm taken from the other.

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[1] *Spemann and Schotté*, 1932; *Holtfreter*, 1935b.

Example 68.[1]

> The development and morphology of the oral implements of anurans and urodeles differ very characteristically. The anuran tadpole has horny jaws, while the urodele larva possesses typical calcareous teeth. If, then, body ectoderm of an anuran larva is grafted over the future mouth region of a urodele larva, it takes part in the formation of the mouth of the urodele host; *but the insert derived from the transplanted anuran material has horny jaws instead of teeth* (Fig. 75). The reciprocal result is obtained when urodele ectoderm is employed in an anuran mouth.

In all these cases the grafted materials have evidently reacted very specifically to the local field influences. But in translating these influences into actual effects they have followed their own innate ways and means. Since we have no other method of detecting field effects than by recording their eventual manifestation in the form of differentiated structures, it is impossible to decide whether homologous fields of anurans and urodeles are alike, or whether they are

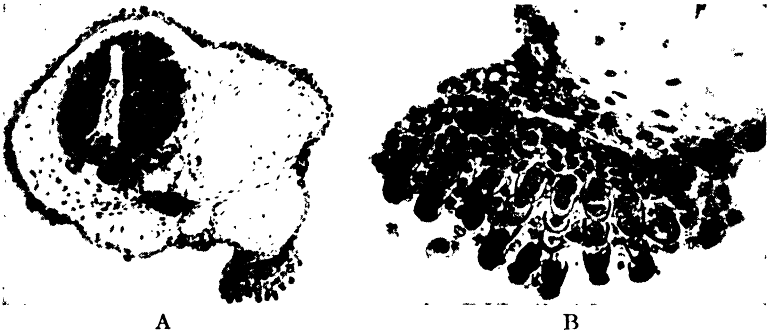

FIG. 75. Specific response of xenoplastic grafts. (From *Holtfreter*, 1935) Indifferent ectoderm of the tree-frog, *Hyla*, transplanted to the ventral head region of the newt, *Triton*, has developed horny teeth typical of the *donor* species and unknown to the host species.

A, Cross section through the host at the level of the graft. × 40.

B, The set of horny teeth of the graft in higher magnification. × 186.

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[1] *Holtfreter*, 1935a.

merely so similar that materials of one group can still respond to the fields of the other. At any rate, *the cells do understand the field "language" of the distant order or family, and they reply to it in their own "terms."*

Field action and cell repertoire.

It remains an entirely empirical task to establish just to what extent the specific character of a given developmental process is due to the specific activities of the field on one hand, and to the specific reactions of the cells on the other. The realization that every developmental phenomenon must be scrutinized in this double light undeniably complicates the concept of development considerably, very much to the regret of the scientist who prefers simple pictures. In order to obtain an organized embryo, there is required, first, an orderly and *specific field action,* and second, a proper and equally *specific reactivity* on the part of the materials called upon to do the actual building. Accordingly, the action of the field and the potencies of the cells must match, like key and lock. Every cell, as we have said above, possesses a definite assortment of "locks," each of which opens a separate course of differentiation; and the local field factors represent "keys" which unlock one or the other, depending on where the cell is located in the field district. For example, the mesenchyme cells of a limb bud, potentially capable of differentiating muscle fibers, cartilage cells, connective tissue, etc., fall under different specific field influences, depending on their location. In the center the conditions will be just such as to unlock that chain of reactions which leads to the production of cartilage; in a more peripheral position, foci of different "key actions" will be established which unlock the cellular mechanism of transformation into muscle, and so forth.

One thing must be borne in mind, however: while ordinary keys are made to fit their locks, the stirring fact about development is that the key actions of the fields have never before in the actual life of the individual been in contact with the lock reactions they evoke. They are pre-fitted and their mutual correspondence, indispensable for the orderly course of development, is simply another manifestation of those pre-arranged and pre-adapted relations among parts of the organism, as well as between the organism and its environment, which are so characteristic of life; embryology can only demonstrate their operation, but cannot account for their existence. To explain the latter, recourse must be taken to principles transcending the history of the individual, such as, for instance, adaptive evolution (see also pp. 457, 558).

Is inductive field action necessarily specific?

Undoubtedly, it is an uncomfortable thought to the analytical embryologist that he must take these pre-arranged affinities and relationships for granted, as something lying beyond the reach of his analytical methods. This sentiment has led repeatedly to attempts to dodge the problem altogether. In the present case these attempts have taken the form of attributing the whole specificity of the patterns of development to the *reacting* materials and relegating the role of the field to that of an entirely *unspecific* impulse which would determine merely when and where the next step in development is to take place, but would have no bearing on the character of that step. It would strike the match, as it were, but have nothing to do with the pattern of the ensuing fireworks. In our preceding discussion a contrasting stand has been taken inasmuch as the field actions were considered as *specific.* This view, however, is not undisputed.

Speaking concretely, it has been questioned whether an eye when inducing a lens, or the chorda-mesoderm when inducing medullary development, are doing anything more specific than giving the adjacent ectoderm an indiscriminate impulse and then leaving it to its own devices. Since medullary plate develops so much earlier than lens, one could argue that the reactive capacities of the ectoderm had in the meantime changed so that the same stimulus which had earlier provoked a medullary response would provoke the formation of a lens when it reappeared during the lens-sensitive phase of the ectoderm. According to this view, the difference in the results would be attributable solely to differences in the *reacting materials* while the assumption of differences among the *acting stimuli* would become superfluous. In favor of this view one can cite some rather suggestive experiments.

Example 69.

Unspecific activation of limb development.

In example 59 we described the induction of a cartilaginous capsule by a transplanted ear vesicle. Only axial mesoderm, however, reacted in this manner. When the graft was so situated that it affected the *lateral* hypomeric mesoderm, then the latter reacted with a local condensation of mesenchyme which in a number of instances developed into a limb (Fig. 76A).[1] In this way the *production of a supernumerary limb can be provoked in the lateral mesoderm* along the flank anywhere between the sites of the fore and hind limbs.[2]

Undoubtedly the grafting of the ear has started typical morphogenetic processes in an area which otherwise would have remained quiescent. But is the ear also responsible for the *specific character* of the development which it has initiated? Apparently not, for the result remains the same if a *nose* primordium instead of an *ear* is grafted to the flank.[3] In fact, even a piece of *celloidin* can produce the effect (Fig. 76B).[4] The induced organ is a *limb* regardless of the kind of graft used. Since lateral plate mesenchyme is the regular source of limb material, one could explain these results very plausibly by assuming that limb-producing capacity is distributed throughout the lateral plate, but remains latent unless it is released by some local stimulus. Obviously, the irritation connected with the presence of the ear, nose, or celloidin graft has furnished this stimulus; it has *activated* the adjacent lateral plate material whereupon the latter proceeded to develop *according to its inherent tendencies.* The stimulus has merely tapped a store of latent limb-forming

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[1] *Balinsky*, 1927a.

[2] *Balinsky*, 1933.

[3] *Balinsky*, 1937.

[4] *Balinsky*, 1927b.

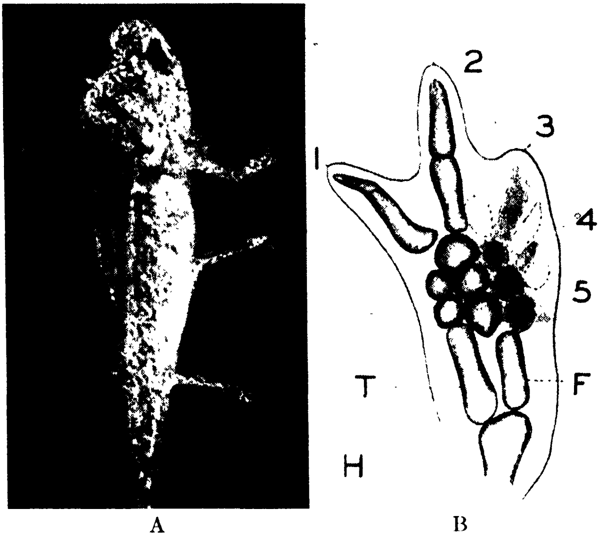

FIG. 76. Activation of extra limb formation by grafts.

A, Transplantation of a nasal placode to the right flank of an embryo of *Triton taeniatus* has provoked the formation of a supernumerary hind limb in the vicinity of the graft. (From *Balinsky*, 1937)

B, Reconstruction of an extra limb developed from the flank in response to the implantation of a piece of celloidin. (From *Balinsky*, 1927)

H, Femur. T, Tibia. F, Fibula. 1, 2, First two toes. 3, 4, 5, Beginning differentiation of the other toes.

processes in an unusual place; in normal development the corresponding stimuli would only be active at the sites of the fore and hind limbs.

Inductions whose character is determined by the host.

Similar results were obtained in other grafting experiments. For example, secondary embryonic bodies produced by the transplantation of presumptive chorda-mesoderm, as described in example 61, were frequently found to be so aligned with the host embryo that corresponding organs of both were lying at exactly the same levels, eyes with eyes, ears with ears, etc. (Fig. 71A, B). This fact has been referred to as the "*regional influence of the host,*" and means that in these cases the induced formations conform with the local character of the reacting host rather than with the character of the inciting graft.

Example 70.

> Transplantation of the *posterior* portions of the notochordal plate [1] or the notochord [2] into *posterior* regions of a host embryo results in the formation of a secondary embryonic body which remains fragmentary and consists largely of trunk and tail, i.e., *posterior* parts. Conversely, *anterior* fractions of the notochordal plate or the chorda, when transplanted into the *anterior* regions of a host larva effect the formation of *head* portions of a fractional secondary embryo. Since the reacting host regions were in these cases exposed to grafts which had come from approximately the same regions of the donors, it is impossible to decide whether the character of the secondary formations was determined by the action of the graft or by the reaction of the host. But a decision could be expected from experiments in which *trunk* portions were grafted into *anterior* parts of the host germs, and *head* portions into *posterior* parts. In a number of such cases the *secondary embryo bore the character of the host region* rather than of the graft, with strict levelling up of corresponding primary and secondary organs (Fig. 71A, B). Thus, inductions provoked by a *trunk fragment* grafted into *head* regions frequently turned out to be *heads.*

This seems indeed to corroborate the results obtained in the preceding example. It looks as if the transplants, no matter where they had been taken from, had merely provided an unspecific impulse to the surrounding host tissue, leaving the character of the formation to be decided by the formative tendencies of the affected locality.

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[1] *Spemann,* 1931; also *Hall,* 1933.

[2] *Bautzmann,* 1929b.

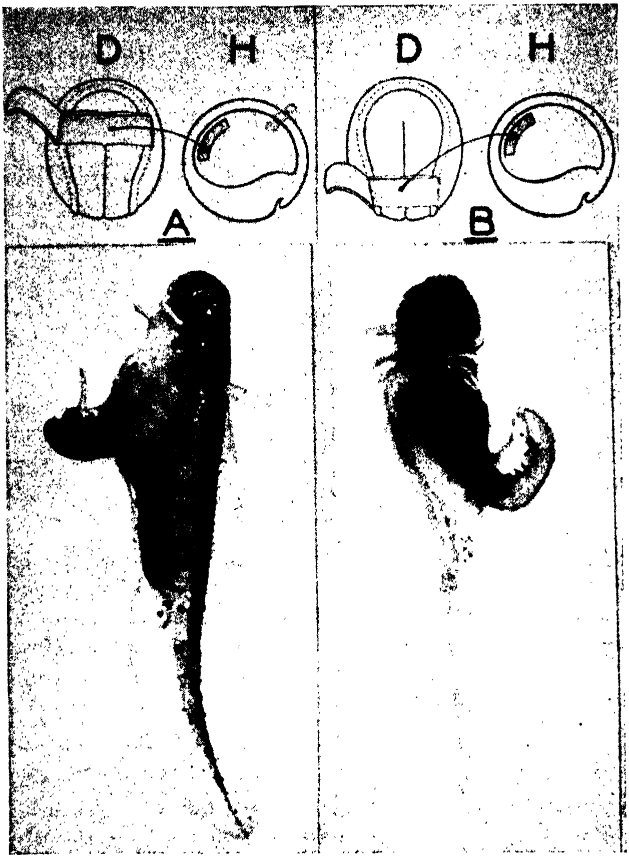

FIG. 77. Graft fields controlling the organization of accessory embryonic formations. *Anterior* chorda-mesoderm causes *head* formation (A); *posterior* chorda-mesoderm, *tail* formation (B). Operations explained in top row: Neurulae served as donors (D), beginning gastrulae as hosts (H). The grafts were inserted into the blastocœle and became wedged between the ectoderm and the invaginating mesoderm of the host during gastrulation. Note that the site of the accessory structures is nearly the same in both cases; their different character can, therefore, be definitely ascribed to specific effects of the grafts. × 15.

Inductions whose character is determined by the graft.

However, all experiments did not take this turn. In fact, in a good number of cases the results were in sharp contrast to the ones just described.

Example 71.

The experimental series reported in example 70 contained a second type of results. Sometimes a posterior *trunk* portion grafted to the *head* region did induce *trunk* structures and, conversely, some *anterior* fragments transplanted into *trunk* levels of the host induced *head* structures there (Fig. 77, 78).

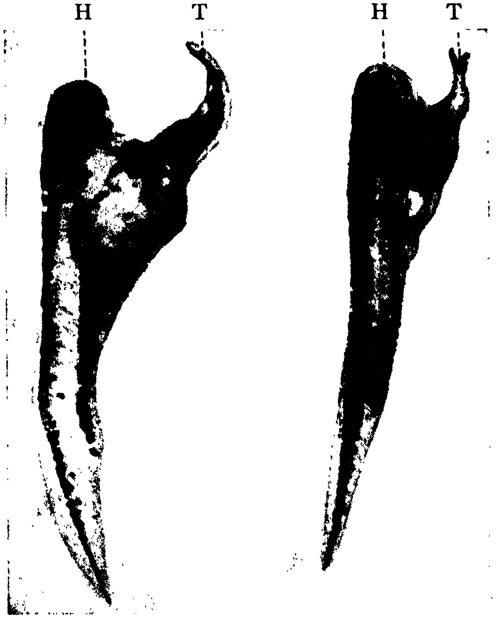

FIG. 78. Graft fields controlling the organization of accessory embryonic formations. (From *Holtfreter*, 1936)

Graft: presumptive *trunk* mesoderm excised at the time of its invagination around the dorsal lip of the blastopore. Host: early gastrula. Location: near head. Result: formation of tails; no correspondence of character, orientation or polarity between the accessory structures and the host embryo.

H, Head of host embryo. T, Secondary tail. × 12.5.

Analogous results were obtained when anterior, middle, and posterior thirds of the medullary plate were transplanted and allowed to act on ectoderm of younger hosts.[1] The inductive capacity of medullary plate was reported above (p. 366). Testing the effects of its different levels, it was then found that the structures induced by them bore positively the *specific charac-*

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[1] *Mangold*, 1929a, 1933b.

ter of the donor region from which the graft had been taken rather than of the host region into which it was put.

These cases prove conclusively that the inducing field does exert *qualitative* and *specific* effects. It is not consistent with the facts, therefore, to consider induction in general as merely an unspecific release action. As we now see, the inducing field can actually determine the character of the provoked organs. How, then, should one reconcile the conflicting results of examples 70 and 71? Evidently, the explanation is as follows.

Field pattern and field energy.

Every developmental process seems to be conditional on two prerequisites: first, an *activation,* that is, something that starts and maintains activity, in other words, some form of *energy;* and second, some *pattern* directing the activities into definite channels. These two conditions are like the fuel and the construction of an engine. The former furnishes the explosive energy, and the latter furnishes the organized conditions which turn a potential blast into an orderly and specific performance. The same fuel, fed into different types of engines, produces different results.

Host field and graft field compete for available material.

We may assume that a transplanted fragment of presumptive chorda-mesoderm contains both prerequisites — an activating or excitation factor (α), and an organizing field factor (β). Introduced into a host germ, the graft, by virtue of α, arouses increased activity in adjacent host tissues. As for the direction which these activities will take, there is the alternative of following either the field of the graft or the local field of the host. If both are of the same kind, no conflict arises. If they are different, however, they compete for control of the activated material. The stronger field will prevail and prevent the weaker one from having any effect. It will depend on the accidents of the operation, on age, size, origin, and location of the graft whether its field influence will or will not be strong enough to overcome the competition of the host field. If the graft field succumbs, the result will be that of example 70, showing so-called "regional influences of the host"; if the graft field prevails, the result will be of the kind described in example 71. Although this interpretation proves adequate to reconcile otherwise wholly incongruous facts, nevertheless, it would remain a mere expedient, were it not that evidence from other sources speaks strongly in its favor.

One of the main supports comes from the fact that it has been possible to separate experimentally the activating factor (α) from the organizing factor (β). *When a field district is killed, it loses its field properties*, which, therefore, must be regarded as contingent on vital activity, *while the stimulating and activating capacity can be retained.* Accordingly, inductions obtained by *dead* implants, such as the limb induction by celloidin described above in example 69 and a number of others which will be mentioned on a later occasion (p. 383), owe their specific character solely to the local host field in which they arise. But it is different with *live* grafts; in addition to activating the host tissue, they can also direct the developmental course of the activated tissue.

FIELD ENERGY AND FIELD PATTERN

The Gradient Concept

It seems fitting in this place to digress from our main line to examine the bearing of the facts just reported on a widely renowned theory of development which has been among the very first to advance a rational interpretation of the *dynamic* character of development, namely, the theory of *physiological gradients.*[1]

The gradient concept has its main roots in observations on regeneration in lower animals. Many lowly invertebrates, such as hydroids, flatworms, annelids, nemerteans, can repair extensive losses of their bodies by regeneration. When an individual is transected, each fragment reproduces the missing portion, retaining its original *polarity,* that is, the anterior piece grows a new tail, and the posterior piece grows a new head, or whatever corresponds to head and tail (e.g., hydranth and stolon in hydroids). Only the extreme tips of the body behave exceptionally in that they reproduce themselves instead of the removed complementary portions. Intermediate sections of the body, however, bounded by an anterior and a posterior cut surface, regenerate a head at the former and a tail at the latter.

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[1] *Child*, 1928, 1929a.

Intrinsic polarity based on gradients of activity.

What is it that gives every fraction of the body cut out at random such a definite sense of direction as to what is "anterior," and what "posterior" with regard to what used to be the intact body? Geometrically speaking, "anteriorly" and "posteriorly" mean pointing toward the head end and tail end, respectively. Therefore, once head and tail have been removed, what criterion of anteriority and posteriority would there be left by which a mutilated fragment could ascertain its former orientation? Obviously, each fraction of the body possesses some inherent polarity of its own. This has been called *physiological polarity.* A railroad car has no intrinsic polarity of this kind; its ends change their designation of anterior or posterior with the position of the engine. Every segment of a worm, however, has inherent polarity, which means that the end pointing headward — toward the "engine" — is in some manner definitely distinguished from the end pointing tailward, even though there may be no visible counterpart to this distinction.

The most convincing exhibition of this polarity can be seen in the fact that each fragment regenerates head and tail in their correct directions. It was left to the work of *Child* and his school to show that this "sense of direction" in body fragments has a tangible and demonstrable basis in a "physiological gradient" pervading the body. Comprehensive studies have revealed that, as one proceeds along the axis from the anterior to the posterior end of one of the cited lowly animals, certain physiological properties decline and increase again in a regular gradation. The rate of respiration, for instance, as measured by the rate of oxygen consumption, is greatest near the front end, decreases gradually in an antero-posterior direction, and finally climbs again to a second, though lower, peak near the rear end. Taking oxygen consumption as a measure of aerobic metabolism, the data suggest the existence of a *gradient of metabolic activity*, declining in a general antero-posterior direction, with a minor reversal toward the rear.[1] Other physiological properties were found to follow a parallel course.

The mere fact that physiological gradients of one kind or another exist, cannot be denied. Whether they are primarily quantitative *metabolic* gradients, is a different matter.[2] At any rate, there seems to be a common metabolic denominator to the various types of gradients that have been observed. This is obvious in the cases of direct or indirect (by indicators) determinations of oxygen consumption and carbon dioxide output; but it is likewise suggested by the gradients of susceptibility, tolerance and recovery, paralleling in general the respiratory gradients.[3]

Gradients of susceptibility.

As we have already outlined on an earlier occasion (p. 159), toxic or otherwise injurious agents do not affect all parts of an organism to an equal degree. The more sensitive parts suffer first and more severely than the less susceptible ones. Depending on the dose of the irritant, the damage may be reparable or permanent, and, in the latter case, range from simple retardation to full destruction (autolysis). This provides us with a scale by which we can estimate the severity of the effects. Thus, by exposing an animal to a

[1] *Child and Hyman*, 1926.

[2] See the criticism of *Needham*, 1931.

[3] *Child*, 1928.

sublethal dose of one of the harmful agents, the "*differential susceptibility*" of different regions of the body can be determined. Experiments of this kind have revealed that the susceptibility of organisms of relatively low degree of differentiation varies along the axis of the body in the same sense as the axial gradient of metabolic activity. That is, the susceptibility is highest at the front end, diminishes caudad, and mounts again toward the hind end. The nature of the agents through which the harmful effects are produced is, on the whole, irrelevant; chemical means (asphyxia, narcosis, poisoning, changes in the composition of the milieu) as well as physical actions (ultraviolet radiation, extreme temperature) are equally effective and produce comparable results. Weak irritations can be followed by recovery, and, again, the rate of recovery varies for different levels of the organism in accordance with the gradient. The same is true for the differential "acclimation" to slightly harmful agents of which different portions of the body are capable ("differential tolerance").

The striking parallelism between the gradient of susceptibility and that of metabolic rate has led to the contention that increased metabolic activity implies greater sensitivity, as well as a higher rate of recovery. Whether this is strictly true or not, the experiments certainly demonstrate convincingly the existence of graded differentials, that is, "gradients," of some sort along the axes of the examined forms of animals. If, in order to avoid unconfirmed implications, we designate these gradients as "*physiological* gradients" rather than as "*metabolic* gradients," we can safely consider their existence as an established *fact*. But does this mean that their role as *factors* of development is equally well founded?

The relation between physiological and morphological polarity.

The theory favored by *Child* is that gradients actually are *instruments* of development. This contention is primarily based on the observation that conditions which alter or obliterate the original polarity of regeneration are attended by a similar disturbance of the axial gradient. It is possible, for instance, to cause the apical fragment of a transected hydroid to regenerate from its posterior cut surface another apical end with a hydranth, instead of a basal end with a stolon. Thus bipolar, and even multipolar, forms can be obtained.[1] In many of these cases the formation of a new morphological axis has been noted to go hand in hand with the establishment of a new gradient, as revealed by one of the methods cited above. Furthermore, it was assumed that when a fragment starts to regenerate, the decision as to where the head and where the tail are to be produced, depends on the direction of the gradient, inasmuch as the "high" end of the gradient would promote the formation of a head; the "low" end, then, having second choice, as it were, would have no other alternative left but to produce tail. As a matter of fact, the coincidence between head development and the "high" end of the gradient has been demonstrated in numerous instances.[2]

All these facts, however, show only that there is an intimate *correlation between physiological and morphological polarity.* In no way do they prove that the former is actually the *cause* of the latter. If someone should, therefore, prefer to consider the polar differences of respiratory rate as collateral manifestations and by-products of the morphological differentiations rather than as their cause, there has nothing thus far come to our knowledge that would contradict his view.

The gradient concept in embryogenesis.

The gradient concept, originally based on studies of regeneration and reconstitution, has been extended to include *embryonic* development. Certain observations encouraged this generalization. It was noted, for instance, that those regions of a germ or embryo in which development makes particularly rapid progress exhibit also relatively high metabolic activity.[1] The differential susceptibility method detects embryonic centers of locally increased metabolism quite readily; so does the differential reduction of vital dyes. In this manner, what might be called the "metabolic pattern" of the embryo has been established. It seems to correspond in gross lines to the pattern of developmental activities. That is, centers of greater developmental activity are said to be characterized by higher rate of metabolic activity, and when the former subsides, the latter declines, too. *Increased developmental activity, therefore, seems to imply increased metabolic rate.* But again we must ask: Is it admissible to reverse this statement and claim, as has been done on occasions, that gradients of metabolic activity *generate* developmental activity? So far as one can judge from the existing evidence, this thesis, which converts the metabolic gradient from a by-product into a primary factor of development, is still an unconfirmed hypothesis.

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[1] *Child*, 1927, 1931.

[2] E.g., *Watanabe*, 1935.

Even though the developmental effectiveness of purely *quantitative* differentials of metabolism which the gradient concept emphasizes is, thus, in doubt, the possibility that different and specific *types* of metabolic processes may perhaps have specific effects on differentiation deserves serious consideration. Our usual preoccupation with the quantitative rather than the qualitative aspects of metabolism is largely to be blamed on the methods employed in our studies in which we measure the balance sheet of the total metabolism without discriminating between its component phases which are possibly quite diverse in character. This diversity, however, the "gradient" methods cannot divulge; just as an ordinary photographic plate can only register the shades, but not the colors, of objects. Although qualitative differences of metabolism have been mentioned in connection with the problem of embryonic differentiation,[2] concrete investigations into the matter are still very scarce.

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[1] *J. Brachet*, 1934; *Brachet and Shapiro*, 1937; *Rulon*, 1935.

[2] *Woerdeman*, 1936; *Needham*, 1936; *Whitaker*, 1937.

Growth following irritation.

The question whether metabolic gradients are or are not causative agents of development must be cleanly divorced from the well established fact that local irritations of various kinds can incite local growth and differentiation and that there may be a concomitant rise of metabolic activity. Activation of local development under the influence of an external stimulus has been illustrated in examples 69 and 70. An additional example is described in the following:

Example 72.

The apical end of the hydroid, Corymorpha, carries a hydranth with a double crown of tentacles, while the basal end is developed into a stolon. Amputation of the apical portion of an animal at practically any level is followed by regeneration of a new hydranth. On the other hand, if the side of an animal is slashed without removal of the hydranth, the wound usually heals over smoothly and nothing further happens. Yet one can provoke the outgrowth of a new stem and hydranth from such

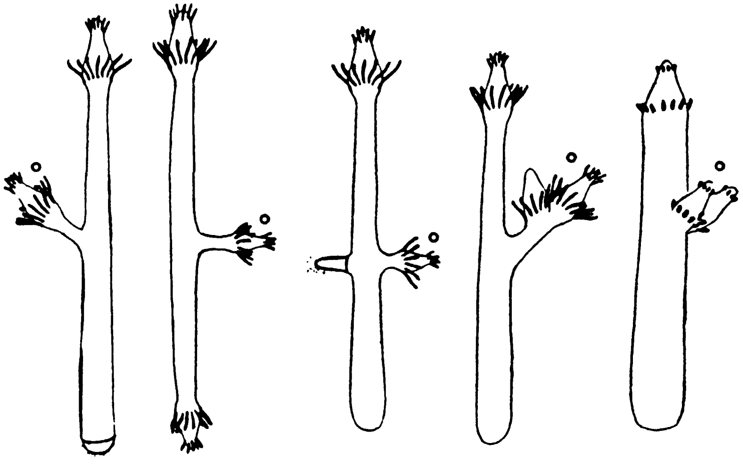

FIG. 79. Regeneration of lateral hydranths in response to strong injury of the body wall in the hydroid, *Corymorpha.* (From *Child,* 1927) The newly formed lateral hydranths are marked by a circle.

a lateral wound, if instead of making a smooth incision, one lacerates the body wall so as to cause considerable irritation. In this case, a bulge forms at the wound which later can differentiate into a more or less perfect hydranth (Fig. 79).[1] There is, however, an obvious tendency on the part of the old hydranth to suppress the establishment of a second hydranth in its vicinity. This inhibitory effect is expressed in the fact that supernumerary hydranths can be evoked the more easily, the greater the distance from the original hydranth becomes; also, the stronger the irritation has been. A fragment of another stem grafted into the wound is more potent in establishing lateral outgrowth than is mere laceration. This result duplicates closely the formation of an extra limb provoked by the irritating influence of a live or dead graft reported above (example 69). In the present case, however, a direct relation between the formative power of the new center and its level of metabolic activity has been suggested.[2]

In analyzing these and similar experiments, we recognize the following points. Irritations of a proper kind produce local formative activity. Such fresh activities, however, are faced with the antagonistic influence of the active structures already in existence. They can superate this resistance and establish themselves permanently, if they are sufficiently strong from the beginning. If they are too weak, they are suppressed. Their "strength" is possibly reflected in corresponding degrees of metabolic activity. But again it is impossible to decide whether the rise of metabolism is a primary or secondary effect. For, any increase, no matter how it is produced, of cellular activity, migration, and proliferation is likely to be associated with an increased turnover of energy.

Finally, as a last comment on this subject, it must be stressed that even if gradients were factors of development, they could serve only as *activating* but not as *determining* agents (see p. 186). If one compares, for instance, examples 69, 70, and 72, one notes that they have in common the action of a local irritant, followed by a definite tissue reaction, accumulation and proliferation of cells, and eventually the shaping of these cells into a definite structure. *What* structure, however, depends entirely on the *organizing capacities residing in the affected locations* rather than upon the *activating stimulus.* There can be no mistake about this fact. The gradient concept states, for instance, that at the "high" end of the gradient head formation takes place, and at the "low" end tail formation. But in this whole interpretation "head formation" and "tail formation" are treated as if they were elemental entities in need of no further analysis, while in reality the terms "head" and "tail" include the whole problem of organization in unsolved form. Thus the cited explanation, instead of elucidating the problem of organization, merely equates it with itself. For, the organization of a head or of a tail is neither less perplexing nor less obscure than that of a whole individual.

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[1] *Child*, 1929b.

[2] *Child*, 1932.

Gradients as necessary attributes of activity.

On the positive side, however, the usefulness of the "gradient" as an *indicator* of dynamic agents must be fully acknowledged, and the merit of the gradient doctrine in having stressed the dynamic aspects of development can hardly be overrated. Any activity maintained from a localized center, whether physical or chemical in nature, must decline in intensity with increasing distance from the focal point.[1] Radiations, electric fields, mechanical tensions, chemical diffusions — all these decline in density or intensity along centrifugal gradients from the source, inversely as the squares of the distance in the simplest cases. In view of the fact that developmental processes obviously operate through physical and chemical forces, it is not at all surprising to find all developmental systems in their active states displaying gradient characters. Thus, as one moves from one center of developmental activity toward another, there is first a steady decline, until one arrives at a trough which marks the region in which both activities are of equal strength, and from there one ascends the slope leading to the other center. This is precisely the form of the "axial gradient" in worms and hydroids (see p. 375); but it is also the picture we obtain when we proceed from the focus of the animal field to that of the vegetative field in the sea urchin egg (pp. 276, 279), or from one local field of organ development to another in the amphibian embryo (see p. 317). *Gradients* of one sort or another are, thus, *necessary attributes of activity*, and we must regard their existence as one of the fundamental proofs of the *dynamic* character of development. Gradients reveal activity. Therefore, we concur with those tenets of the gradient doctrine which deal with developmental activities *as such*, detached from their hypothetical metabolic interpretation. We concur with this doctrine in stressing that the transformation of the germ into the developed individual is not a frictionless and static separation of independent parts, but is the result of competitive interactions of mutually interfering parts asserting themselves in proportion to their actual strength and outlining their mutual boundaries accordingly.

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[1] Providing the action is not of the relay type.

Summary: A dissection of the gradient concept.

As can be seen from this discussion, the gradient doctrine is not an indivisible whole, but is composed of independent propositions some of which can be accepted, while others may be rejected. They can be summarized as follows.

Developmental activities in the germ differ locally. That the differences are *merely* quantitative, mere variations of intensity of one and the same process, more specifically, of a single kind of metabolism, has never been proven. That there are *also* quantitative gradations, follows from the gradient character commonly attaching to physical and chemical activity. Increased tissue activity seems to require increased metabolism. Therefore, gradients of developmental processes may be accompanied by gradients of metabolic activity. The existence of such gradients can be taken for granted. They are *indicators* of developmental activity.

What, then, produces these local differences in germs of which gradients are an expression? External factors with concurrent gradients? Local irritation, as we have seen, can provoke formative activity, and this is often associated with a local rise in metabolism. Whether this rise is instrumental in provoking growth and differentiation, or merely coincidental, has not been decided; again, one should consider not only the rate of metabolism, but also its character, a point on which we lack basic information. Whatever the primary effect of the irritating stimulus may be, however, it has no bearing on the *pattern* of the developmental processes which follow. Although these processes may derive their *strength* from the metabolic effects of the primary stimulus, their *character* is determined by the latent formative tendencies (fields) of the irritated region.

Pure Activators

Separation of the activating and pattern determining principles.

This discussion has reaffirmed our earlier statement that every act of development requires, first, the *unspecific activation of energy,* and, second, a *specific pattern* to direct the resulting activities. We have made greater progress, thus far, toward an understanding of the activating than of the organizing principle. We have learned to duplicate the activating effect of the sperm by non-living agents (artificial parthenogenesis), and to evoke formative processes through artificial stimuli. Thus hydranths were elicited by mechanical trauma (example 72), and limbs by the insertion of celloidin into the body wall (example 69). But far more spectacular effects were obtained in the following examples.

Example 73.

When an amphibian germ is killed by heating, boiling, freezing, or drying, and parts of it are then implanted under the ectoderm of a normal gastrula, the *host ectoderm reacts by producing extensive supernumerary formations* (Fig. 80).[1] Brain, nose placodes, eyes, lenses, ears, ganglia, balancers, and mesenchyme have been identified in these "inductions." Implantation of fragments of germs killed by chemical agents, such as acids or narcotics, has the same effect. Finally, dead tissues from all sorts of stages and classes of animals were tested, and the more one examined, the less logic there seemed to be in the results. Tissues ineffective in the *living* condition, would give induction effects after they had been *killed*. *Adult* or *larval* tissues worked as well as *embryonic* ones; body fragments of killed *worms*, *snails*, *crustacea*, and *insects* could produce as good an effect as could the tissues of *vertebrates* (Fig. 81).[2]

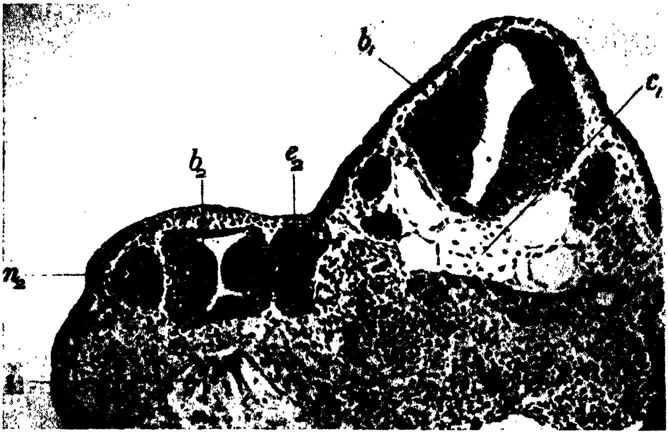

FIG. 80. Secondary embryonic structures provoked by the implantation of dead tissue. (From *Holtfreter*, 1934) Operation: implantation of alcohol-fixed blastoporal lip of an Axolotl germ into an Axolotl gastrula (head region). Result: formation of supernumerary head organs (brain, 2 noses, epiphysis, ear) of which the section shows brain (b_2), nose (n_2) and eye (e_2; note black line of tapetum). Note the absence of a lens in the eye due to lack of contact between retina and epidermis (see p. 332). i, Implant. c_1, Notochord of host. b_1, Brain of host. × 56.

In all these cases the implanted piece must have contained some activating principle which has *called forth strong*

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[1] The most extensive experiments in this field have been performed by *Holtfreter*, 1933c, 1934a; additional literature reviewed in *Weiss*, 1935b.

[2] *Holtfreter*, 1934b. Recently, *Okada* (1938) has demonstrated the activating capacity of *inorganic* implants (Fuller's earth, silica, calcium carbonate).

developmental activity which otherwise would not have occurred. The role of this principle, however, has been confined to stirring up the host tissue. What further happened to the activated tissue, was wholly *an affair of the host field;* consequently, the resulting formations bore the regional character of the host (see p. 369). Not all agents are equally potent. Some evoke extensive organ formations, while others, under the same circumstances, incite only very abortive tissue reactions.

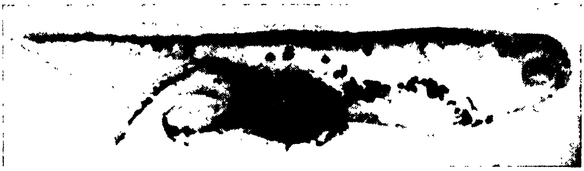

FIG. 81. Provocation of supernumerary formations by implantation of strange substances. (From *Holtfreter*, 1934) Implantation of a piece of fresh mouse kidney into a gastrula has called forth secondary embryonic fragments of which the two tails seen in the picture are most prominent. The interior contains also some extra brain and musculature. × 13.

Chemical activators.

The wide and indiscriminate distribution of the activating principle throughout the animal kingdom indicates that it is of rather elemental order, and it was not far-fetched to conjecture that it might consist of some *chemical substance.*

Example 74.[1]

One proceeded to make extracts in various solvents from the tissues whose inductive power had been ascertained. After purification, the extracts were taken up into some carrier substance with no inductive capacities, such as, for instance, agar, and then were introduced into gastrulae by the usual grafting method. While some extracts were absolutely ineffective, others provoked *reactions of the host embryo* culminating in medullary formations. Again it must be stressed that the character of the reaction was determined by the reacting tissue, not by the activating stimulus.

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[1] This work in which numerous workers took part has been reviewed by *Needham*, 1936.

A chemical identification of the effective extracts was possible within certain limits. Related substances of known composition, sometimes synthetic compounds, were then tested and gave similar results. However, *no one single substance, or class of substances, has been found to possess a monopoly on these effects.*[1] Of course, a chemical substance, as such, can produce no developmental result; to become effective, it must either liberate energy by its own decomposition, or combine with some other substance in the form of a chemical reaction. In the latter case, the effect depends upon the properties of both the introduced foreign substance and the substances present in the body which react with it. Thus, the introduction of the foreign substance presumably initiates a whole chain of chemical transformations, and the actual tissue activation which has developmental significance possibly goes to the credit of some later link in this chain, rather than being a direct product of the inserted chemical. But we do not even know what is the crucial factor, the chemical change as such, or the energy liberated by it. A connection with *specific metabolic processes* has been suggested,[2] but the whole subject is still much too little explored for a final decision. To judge from the positive effects of dead grafts (example 73), the principle is contained in every part of a germ; but its *active* state is in the living confined to the chorda-mesoderm district (see also example 70), while the other parts contain it in an occult or blocked form from which it is only released after death.

Chemical activators in normal development.

Of course, the question arises whether and to what extent such activating agents intervene in the course of *normal* development. All experimental evidence seems to confirm the fact that the presumptive chorda-mesoderm, in being rolled under during gastrulation, dispenses an activating principle to the overlying ectoderm and thus initiates the transformation of the latter into a medullary system. Therefore, at least one essential phase of embryonic development is clearly dependent on special activation. One is tempted to generalize, and to assume that *the activating principle intervenes recurrently,* initiating those later local activities which take shape as lens, ear, limb, gill, etc. One could even maintain that without the intervention of an activating principle development might fail to occur altogether, in spite of the presence of fields and all other prerequisites, just as fireworks do not explode unless they are ignited, or, to use our previous analogy (p. 372), as a motor does not operate without fuel. Such a view is conceivable; whether it also is consistent with the facts, only future experience will tell. If substantiated, it would compel us to decompose field action into two principles of essentially different character and origin: *field energy* and *field pattern. Field energy* would be set free by the activating principle. It would cause the tissue to become productive, and determine how much tissue would become so engaged. What form the production would take, would be determined by the *field pattern,* that is, the set of organized conditions controlling the spatial distribution and the histological differentiation of the activated material.

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[1] *Woerdeman*, 1936.

[2] The cells turning around the dorsal lip of the blastopore lose glycogen (*Woerdeman*, 1933; *Raven*, 1933; *Heatley and Lindahl*, 1937; not confirmed by *Pasteels*, 1936), which, together with the high rate of carbon dioxide output (*Brachet and Shapiro*, 1937; *Fischer and Hartwig*, 1938) seems to signify profound changes in the carbohydrate metabolism of this region (see the reviews by *P. Weiss*, 1935b; *Woerdeman*, 1936; *Whitaker*, 1937).

Coincidence of the sources of field pattern and field energy.

Activated energy without field pattern remains chaotic; dead parts can activate (examples 73, 74), but, having lost their field properties, they cannot establish organization (see p. 373). In the living body, however, there seems to be a regular *conjunction* between the emergence of field patterns and sources of energy to put them into effect. This is especially obvious in examples 71 and 39, which demonstrate the organizing effects of chorda-mesoderm in amphibians and of vegetative fragments in echinoderms. In both cases the graft has supplied both activation and controlling field. The fact that both principles are combined in the same district, easily obscures their essential duality. Therefore, it was a fortunate find when a more favorable object was discovered in which the centers concerned with primordial activation and primordial organization are spatially separated. This object is the *insect* egg.

Organization in Insects

The insect egg as exception.

The interior of the insect egg contains a large amount of yolk. The nucleus lies in the center surrounded by a small mass of protoplasm. The surface of the egg consists of a layer of yolk-free protoplasm, the *blastoderm.* After fertilization nuclear division sets in and produces in successive, synchronous steps two, four, eight, etc., daughter nuclei, while the cytoplasm, at first, remains unsegmented. The nuclei gradually emerge from the interior and settle in the undivided surface blastoderm in a fairly even distribution. It is only then that cell walls begin to appear. The long axis of the elongate egg indicates the antero-posterior direction of the future embryo. The first distinct differentiation of the blastoderm is a change in shape, arrangement, and rate of progress of that part of the blastoderm which is to become the *ventral* side of the embryo. The cells of this ventral plate play a prominent part in producing the fundamental structure of the embryo both by proliferating internal organs into the yolk mass and evaginating the segmental appendages of the embryo.

Example 75.[1]

It was discovered that in the fertilized egg of some insects (dragon-flies) a peculiar agent spreads from the rear end anteriorly with a measurable speed, and that the intervention of this agent is indispensable for development. *Elimination of this agent suppresses differentiation and organ formation,* although *nuclear multiplication* continues. By thermal coagulation or ultraviolet irradiation (see p. 159) of the rear end, the agent, or rather the source of its production, can be destroyed, and, by ligating off the posterior portion of the egg from the rest, one can prevent the agent from spreading. If the operation is performed early, a small defect is sufficient to prevent further development. But, the later one operates, the more anteriorly one must extend the destruction in order to suppress development. This, together with some other evidence, proves that there is an agent which progresses from the rear in a postero-anterior direction and releases development in its wake. It moves like the crest of a wave. It can be trapped by interrupting the continuity of the egg in front of it; anterior to the break, the body fails to develop. On the other hand, destructions which fall short of catching up with its advancing front merely eliminate development within the destroyed parts, while development anterior to the region of actual destruction continues and produces an *anterior fragment* of an embryo containing those parts which would normally have arisen from the same egg region.

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[1] This work has been ably reviewed by *Seidel* (1936) in whose laboratory most of the experiments were done.

Spatial separation of activating and organizing principles.

There was no tendency of the developing portion to become shaped into a complete, harmonious embryo. Its organization was strictly partial, displaying those parts only which it would have formed had the rear portion been left intact. Consequently, that peculiar agent is not concerned with the laying down of organization in the blastoderm, and its only function seems to be to release for further development parts which have either received their developmental assignments from another source or have contained them from the very beginning in the manner of a strict mosaic. The latter alternative can be discounted, however, in view of the following experiments.

Example 76.[1]

Approaching a dragon-fly egg with a hot needle often causes the appearance of a cleft in the egg. If this fissure is oriented longitudinally, embryos developing from such eggs show more or less extensive reduplication of the anterior or posterior end of the body, depending upon the location of the defect. In favorable cases, complete twins formed. Similarly, after tying off part of the egg, proportionate dwarf embryos were obtained from the reduced mass lying behind the ligature (Fig. 82).

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[1] *Seidel,* 1929a, b.

These results prove that the examined eggs [1] were capable of *regulative* development. This implies that they were at the time of operation *decidedly not in a mosaic condition.* Just as in sea urchin and amphibian eggs, the parts evidently receive their final assignments only after cleavage has already gotten under way. Further experimental analysis revealed that the *organization* of the embryo is controlled by an area at the *ventral* side of the blastoderm, which, as we have said before, is remarkable for the precociousness of its development. The *field pattern* must be credited to the properties of this region, in combination with the central yolk mass. Contrary to the amphibian and sea urchin germs, however, the field district in the present case merely

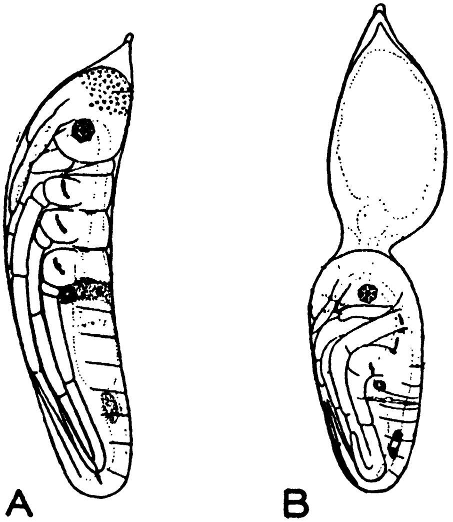

FIG. 82. Development of a whole embryo from the posterior half of a dragonfly egg (*Platycnemis pennipes*). (From *Seidel,* 1936)
A, Normal control.
B, Proportionately reduced embryo obtained by ligating the freshly laid egg around the middle.
Lateral views. Note the completeness of the morphological equipment (eye, antenna, mandible, legs) of the dwarf.

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[1] As will be shown later (p. 410), other insects behave differently.

assigns the parts under its control to specific courses of development, while actual differentiation cannot occur until after that certain activating agent which sweeps forward from the rear end has added its impulse. In other words, the sources of the *activating* and the *pattern determining* principles which in the amphibian and sea urchin germ are combined in a common district, are *spatially separated* in the egg of some insects.

We may, thus, conclude that the organizing (that is, pattern disposing) and the activating (that is, energy supplying) principles are fundamentally two different things rather than merely two aspects of the same principle, although in the majority of cases thus far studied they occur jointly. Their joint presence in the chorda-mesoderm district of the amphibian germ has been demonstrated. If we now turn to the *birds*, we find evidence of an analagous situation.

Organization in Birds

The avian germ.

Most experiments on avian development have been done on the chick egg, in an effort to ascertain the developmental potencies and interactions of different areas of the germ. All three methods commonly used in the study of potency have proved practicable: the simple *defect* experiment, in which part of the blastoderm is destroyed or the connection between different parts of the blastoderm is interrupted; the *isolation* experiment, in which different fragments of the blastoderm are isolated and either explanted in tissue culture or interplanted on the chorio-allantoic membrane of an older egg (see p. 157, Fig. 24); and the *recombination* experiment, in which fragments are recombined in strange orders.

Gastrulation in birds.

In order to compare avian with amphibian development, one must realize the essential homologies between their gastrulation stages. The wide open blastopore of a medium-aged amphibian gastrula is bounded by a circular rim around which prospective mesoderm is rolled under (see p. 341). The corresponding structure in birds, however, consists of a long double ridge, the so-called *primitive streak*, which can be considered as the homologon of the merged lateral lips of a long, drawn-out blastopore. Otherwise, gastrulation proceeds essentially as in amphibians. That is, cells lying in the surface of the blastoderm converge toward the primitive streak, sink down in it, and then diverge again laterally so as to form a mesodermal lining under the surface blastoderm. This movement thus corresponds to the rolling under of mesoderm around the lateral lips in amphibians. Only at the anterior end of the streak, where the merged lateral lips bend over into each other, there is some trace of a real blastopore in the form of a slight depression, called the *primitive pit*, which is overhung by a knob-like structure corresponding to the *dorsal lip* of the amphibian blastopore. This structure, known as *Hensen's node*, proliferates anteriorly under the surface and thus forms the *notochordal plate*, known during early stages as the *head process*. The node moves backward along the ridge of the primitive streak, and in its wake medullary plate, medullary folds, and segmented somites make their appearance.[1]

Defect experiments concerning the role of the node.

Defect experiments, as well as isolation experiments, have shown conclusively that the region of the node plays as prominent a role in the development of the chick as the chorda-mesodermal field plays in the development of an amphibian. Simple defect experiments prove that *some contribution of the node region*, material or dynamic, *is indispensable for the development of more peripheral regions of the germ.* For this reason, parts of the blastoderm separated from the node through a cut fail to develop properly.[2] The defect experiment does not indicate, however, whether the contribution consists of formative materials, or of a releasing activator, or of an actual pattern-determining field influence, or possibly of a combination of these. In conjunction with isolation experiments, however, a fairly coherent picture has been obtained. To view these experiments in the correct light, it is important to remember that isolation experiments test primarily the capacity for self-differentiation of a germinal fragment, and that self-differentiation presupposes, first, viability and an adequate milieu, second, developmental energy, and third, a field pattern. The first condition is met both in chorio-allantoic grafting and in tissue culture (p. 153 f.); as for the second and third, the results vary depending on the age and stage of the blastoderm and on the location from which the fragments are chosen.

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[1] For further details, consult *F. R. Lillie*, 1919.

[2] *Peebles*, 1904; *Hoadley*, 1926b, 1927.

Example 77.[1]

Systematic variation of self-differentiating capacity in interplants.

The most comprehensive test of the self-differentiating capacity of different areas of the chick blastoderm in the stage of a well developed node (head process present) has been made in the following manner. A blastodisc was first divided transversely across the primitive pit into an anterior and posterior fragment. Each of these was then further subdivided into three transverse strips. Finally two sagittal sections, one to either side of the primitive streak, separated the six transverse strips each into a median and two lateral portions. Each of the eighteen different fragments into which the blastoderm had thus been parcelled up was then transplanted to the chorio-allantoic membrane of a separate host. Eight or nine days later the site into which the graft had been placed, was studied. It was found that not all grafts had taken, and that the return of positive takings, as well as the amount of growth in them, varied according to the region from which the graft had come. The largest percentage of successes, as well as the greatest volume of growth, was obtained from grafts which *included the node. With increasing distance from the node the returns diminished* (Fig. 83). The chance of a fragment to survive, to become incorporated, and to continue to grow, therefore, decreases inversely as the distance between its origin and the node region.

If the ability of the grafts to take hold and grow is a measure of their residual energy, the experiments force us to conclude that *developmental energy reaches a maximum in the node region,* and declines from there steadily toward the periphery. The high metabolic activity of the node [1] corroborates this conclusion. Thus, the chick, too, employs an activator principle — energy controlling, but not pattern determining — of which the node region is the source. As for the pattern determining factors, a study of the type of differentiations produced by the grafts has proved very instructive. Numerous samples of each region were reared as chorio-allantoic grafts, and the differentiations obtained were recorded. The results of these studies can be summarized briefly as follows.[2]

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[1] *Rawles*, 1936.

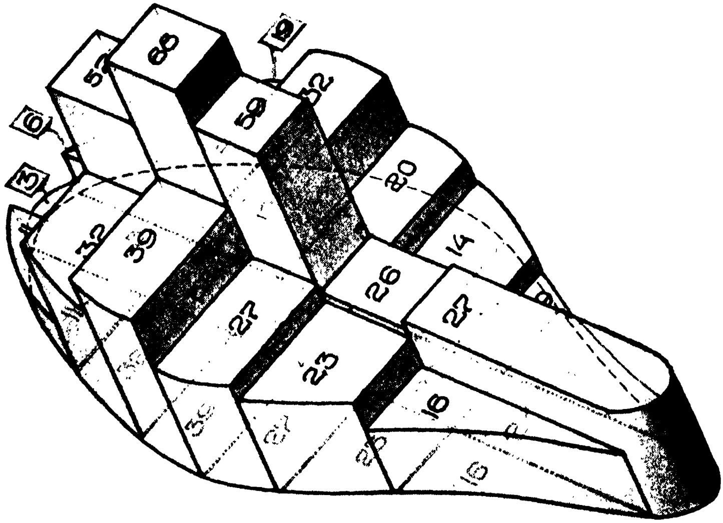

FIG. 83. Stereogram indicating the frequency of "taking" of fragments of 18 different regions of the chick blastoderm when grafted to the chorio-allantoic membrane. (Constructed from the data of *Rawles*) The groundplan represents a blastoderm in the primitive streak stage, divided into 18 parcels. The height of the towers erected on each parcel indicates the percentage of grafts of that area which have survived and differentiated; the numerical values of these percentages are recorded on the floor and top of each tower. Note the all-around falling off of these values from the peak at the node (see text).

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[1] *Rulon,* 1935.

[2] *Willier and Rawles,* 1935; *Hunt,* 1932, 1934; *Rawles,* 1936; *Rudnick,* 1932, 1935; *Stein,* 1933.

The progress of organization in the blastoderm.

First, identifiable structures develop from practically every region of the blastoderm from primitive streak stages on. *Morphogenetically,* that is, with regard to shape and arrangement, development on the chorio-allantoic membrane is distinctly *defective. Histogenetically,* however, that is, in those points which concern the differentiation of cells and tissues, development on the chorio-allantoic membrane is surprisingly *normal.*

Second, complex *organ* formation is confined to those fragments which come from within a certain range around the node. The most peripheral portions of the blastoderm, particularly the anterior and posterior ends, furnish merely unorganized *tissues,* such as epithelium, muscle, and skin.

Third, fragments coming from the immediate vicinity of the node can differentiate practically every organ that ever occurs in an embryo. Samples chosen at some distance from the node form only a certain selection of organs. Each organ has been recovered from within a rather liberal range; but there is always a central region in any such range in which that particular organ forms most frequently and with the greatest amount of perfection. These *organ-specific areas,* therefore, have again the character of *fields.* Figure 84 shows the extent and gradation of the eye, heart, and mesonephric fields at the streak stage. It must be borne in mind that this map does not express the prospective fates which the different portions of the blastoderm would undergo in normal development, but their *potencies,* as manifested in isolation.

Fourth, it can be recognized that *each area has by far greater potencies than it manifests in normal development.* So, for instance, epiphysis, which normally forms in the mid-line, has also been obtained from lateral fragments which contained none of the presumptive epiphyseal material. Conversely, ear and mesonephros, normally bilateral formations, can be obtained from the median strip of blastoderm. Eye can appear in any fragment of the shaded transverse strip (Fig. 84) near the anterior end of the head process.[1] Heart can be produced from practically any level within the limits in which organ formation is possible.

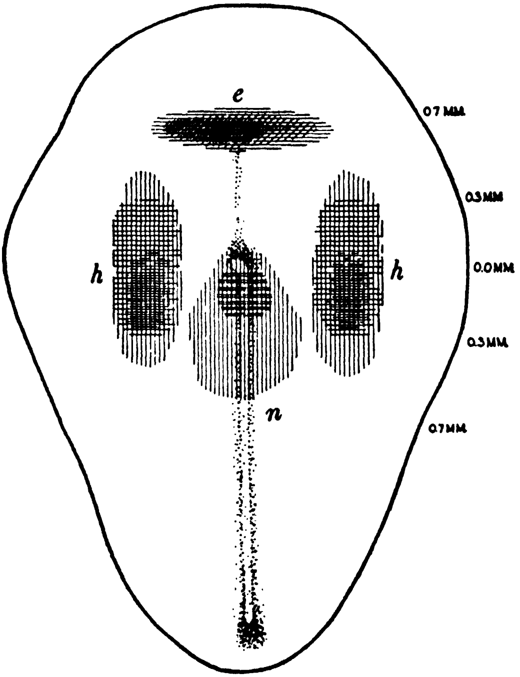

FIG. 84. Organ-specific fields in the chick blastoderm. (From *Willier and Rawles*, 1935) The approximate extent of the eye (*e*), heart (*h*), and mesonephric (*n*) fields in the head process stage of the blastoderm is indicated by the shaded areas. Fragments from anywhere within these areas, when raised in isolation on the chorio-allantois of an older host embryo have differentiated the indicated characters.

Fifth, with increasing age of the embryo, there occurs a *progressive restriction in the formative capacities* of the various areas.[2]

Sixth, mutual specific influences among developing structures are indicated even in the chorio-allantoic grafts. Thus, for instance, the differentiation of liver tissue seems to require the presence of heart,[3] but not vice versa, and the development of medullary plate seems to depend on the head process.

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[1] *Clarke*, 1936.
[2] *Rawles*, 1936; *Rudnick*, 1935, 1938a, b.
[3] *Willier and Rawles*, 1931.

The fields of the avian germ.

These results point toward the following conception of embryonic organization in the chick. The blastoderm, at the end of the primitive streak stage, appears composed of a number of *partially segregated local field districts crowded about the node region.* Each field district for eye, ear, liver, mesonephros, lung, etc., possesses a center in which self-differentiating capacity for that particular organ is most firmly established. Toward the periphery of each field, and as one approaches the domain of other fields, the fate of a given fragment becomes less and less predictable, until greater definiteness is again reached by moving closer to the center of the next adjoining field. Since the overlap between neighboring fields is very wide, one must again postulate that normally each field is endowed with only a limited amount of energy so that it cannot encroach upon materials required for use in neighboring fields. Up to the head process stage, however, no further subdivision of tasks has apparently occurred within these localized field districts. That is to say, none of the individual cells have yet been assigned to any definite differentiation. At consecutively later stages the variety of organs which a given region of the blastoderm can produce shrinks rapidly, which indicates the *gradual progress in determination* made by the materials under the action of their local fields. In this respect, too, the avian germ and the amphibian germ are strictly comparable. In both cases, however, the precise manner in which the *segregation* and gradual consolidation of local fields takes place, is obscure, and little is known beyond the mere fact that they do occur. A little more light has been cast on the phenomenon of induction, that is specific determination effected through *adjoining* structures.

Phenomena of induction in birds.

In the chick, too, contact of the eye vesicle can induce the formation of a lens in other than the normal lens-forming ectoderm[1] (p. 309). Similarly, nasal organs have been induced in ectoderm in contact with the anterior part of the medullary plate.[1] Finally, induction of medullary plate in non-neural ectoderm through the axial structures of the embryo has also been obtained, as follows.

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[1] *Danchakoff*, 1924; *Hoadley*, 1926a; *Alexander*, 1937.

Example 78.[2]

> The primitive streak of chick embryos was tested for its inductive capacities. Fragments of the streak were transplanted into blastoderms of host embryos which were, for convenience, removed from the yolk and cultivated on plasma clots in vitro.

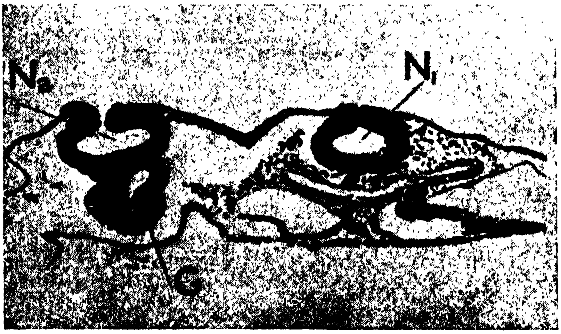

FIG. 85. Induction of medullary tube by a heteroplastic graft in fowl. (After *Waddington and Schmidt*, 1933)

Host: Blastoderm of a duck incubated for 26 hours and explanted to the surface of a blood plasma clot in vitro.

Graft: Anterior portion of the primitive streak, including the node, of a chick embryo incubated for 15 hours.

Result: After 31½ hours of cultivation the host has developed a second neural tube (N_2) in contact with the graft (G), in addition to the primary neural tube (N_1). × 82.

> While the transplants continued to self-differentiate, host ectoderm in contact with them often gave rise to more or less perfect medullary formations (Fig. 85). Even ectoderm of the extra-embryonic area responded to the inductive stimulus. As in the amphibians, the stimulus proved to be non-species-specific, and cross grafts between chicks and ducks proved successful.[3]

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[1] *Waddington and Cohen*, 1936.

[2] *Waddington*, 1933, 1934; cf. also *Hunt*, 1931.

[3] *Waddington and Schmidt*, 1933.

Although inductive capacities have thus been shown to exist in the bird embryo, the actual extent of their occurrence and importance in normal development has not yet been established. As in all similar cases, one must bear in mind that the very fact of isolation puts an excised part of a germ into a position to display activities which in normal development would have been the concern of a much larger part of the egg, namely, that part covered by the whole field district of which the isolated part represents only a fraction.

Organization in Fishes

The germs of fishes.

The *fishes* also fall in line with the amphibians; they display the same phenomena of local field segregation and induction with which we have become familiar from the studies of amphibians and birds. The reason that the order of our discussion — from amphibians over birds to fishes — so obviously disregards the evolutionary order, is purely historical, in that teleosts were the last forms to be studied.

In some respects the teleost egg assumes an intermediate position between the amphibian and the avian egg. The proportion of yolk in it is considerably larger than in amphibians, thus causing partial cleavage and meroblastic formation of the embryo, while, on the other hand, the initial disproportion between the embryonic and the yolk-containing extra-embryonic area is less extreme than it is in the chick. The blastoderm, which develops on top of the yolk, increases by continued cell division and gradually envelops the yolk, but only a circumscribed part of it, the so-called embryonic shield, produces the embryo proper. This subdivision of the germ into an embryonic and an extra-embryonic portion again reminds us of the avian germ. As in the other vertebrates, the molding of the embryonic form begins with the invagination of the archenteron, the differentiation of an axial core from the dorsal portion of the invaginated material containing notochord and mesoderm, and the subsequent development of a nervous system in the ectoderm overlying these axial structures.

Potencies of the fish blastoderm.

Just as in amphibians and birds, the presumptive fate of different portions of the embryonic shield could be traced into the later organs of the embryo by means of vital stains, and similarly, individual regions of the shield could be traced backward to the materials contained in the individual blastomeres of the sixteen-cell stage.[1] With this knowledge of the normal developmental course as a background, one proceeded to test the *developmental potencies* residing in different portions of the embryonic shield.

Example 79.[2]

Most of the attention thus far given to the egg of the teleost has centered on the prominent role played by the dorsal axis. This structure which is composed of the materials invaginated around the dorsal blastoporal lip, is essentially homologous to the chorda-mesodermal district of the amphibians. Therefore, one could have expected that it would exhibit the same totipotence of organizing power, if confronted with properly re-

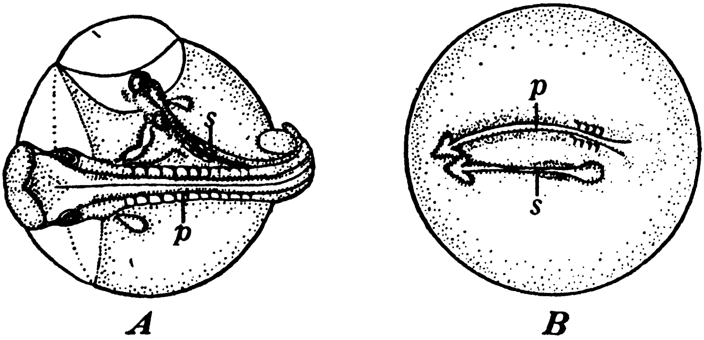

FIG. 86. Induction of accessory embryos in the fish, *Fundulus*. (From *Oppenheimer*, 1936)

A, Secondary embryo produced by the grafting of a dorsal fragment of the blastoporal rim into the edge of a young blastoderm; 3 days after the operation.

B, Secondary embryo produced by a graft of very young dorsal lip into the embryonic shield; 22 hours after the operation. × 22.

p, Primary embryo. *s*, Secondary embryo.

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[1] *Oppenheimer*, 1936b.

[2] *Luther*, 1935; *Oppenheimer*, 1936a.

sponsive materials. As a matter of fact, this expectation was fully substantiated when fragments of the dorsal lip of the blastopore, that is, the invaginating area at the rear end of the embryonic shield, were excised and transplanted heterotopically either into the embryonic shield or into the extra-embryonic blastoderm of another embryo. In either case *secondary embryonic formations were obtained in conjunction with the grafts*, ranging from a few supernumerary organ fragments (e.g., myotomes) to fairly complete and proportionate functional embryos (Fig. 86).

Again the duality of field pattern and field energy.

Obviously grafts from the invagination zone contain both the necessary *activator principle* to stir up host cells of the vicinity so that they become available for new formations, and a *totipotent field* to give the development thus initiated plan and direction. The grafted cells were either harmoniously used along with the host cells, or if they were already too far advanced in their own determination, they continued to self-differentiate. The tissues of hosts of the embryonic shield stage were still widely responsive and complied with the demands placed upon them by the graft field. Just as in the chick, extra-embryonic material was no less compliant than the material of the embryonic shield proper, though extra-embryonic cells under normal circumstances are never called upon to form embryonic organs. This last observation is noteworthy because it furnishes an illuminating example of the utter inadequacy of a strictly preformistic viewpoint of development.

In one point, however, there was a significant difference between the results obtained in embryonic and extra-embryonic regions. In the former case the secondary embryos showed a distinct "*regional influence of the host*" (see p. 369); the induced structures bore the character of the locality in which they were situated. Supernumerary embryos developing in and from extra-embryonic parts, however, failed consistently to show any such relation to the organization of the host. Their character was unmistakably linked with the *regional character of the graft* through which they had been evoked. In order to interpret this alternative behavior, one must only remember the two types of results obtained in amphibians by the grafting of chorda-mesoderm fragments as described in examples 70 and 71. The explanation lies in the double role of the graft which furnishes, first, an activating stimulus and, second, a field pattern. In the embryonic region, the field of the graft must compete with the near-by fields of the host. To judge from the "*regional influence*" of the host, the host fields win. Only in the extra-embryonic region, far removed from the host's own field centers, can the field of the graft assert itself and mold the activated tissues according to its own tendencies.

PREFORMATION AND EPIGENESIS

Reviewing the course of development.

If we are to crystallize the experiences gained from the study of vertebrate and echinoderm development into a common formula, we arrive at about the following conception.

1. At some sufficiently *late* stage of development, organs, tissues, and cells are in the condition described in Part One as characteristic of the developed body: the *structural and functional differentiation of each cell* is, within the limits of modulation, *circumscribed and definite,* and so is the arrangement of cells into tissues and organs.

2. At a somewhat *earlier* stage, the differentiation of specific structural and functional characters is less obvious, and the cells resemble each other to a much greater extent (p. 73). Their arrangement into definitely shaped masses, such as sensory placodes (Fig. 11) or pre-cartilaginous condensations of mesenchyme (Fig. 48), roughly indicates the outlines of the future organs. Testing the developmental potencies of the individual cells at this stage, reveals that their *external resemblance is not an expression of internal*

identity. They are already in possession of definite trends of development and, consequently, differ from older cells merely by the degree to which this definiteness has become translated into manifest recognizable features. Their differences, although *occult,* are *real* at this stage (example 50).

3. *Prior* to this stage, however, *external resemblance among cells* within a given area *reflects true equivalence and equipotency.* In this early stage the cells not only fail to display differences of character but they do not even possess them. At the same time, however, contrasting with the indefiniteness of the *individual* cells, the local *collectives* to which they belong can already self-differentiate in accordance with their presumptive fate. This is the condition in which we encounter the *organ primordia:* eye, ear, limb, mesonephros, heart, liver, etc. (compare examples 52, 53, 55 with examples 28 and 29). At this stage the organism appears as *a mosaic of local areas endowed with specific but restricted fields* directing subsequent development according to their own patterns. Numerous local fields coexist, each engaged in some partial task. But this is not a mosaic in the old sense of peaceable neighbors, going about their business in mutual noninterference; it is a *mosaic of dynamic centers* which lie in active competition, each trying to draw available materials into its sphere of influence according to its strength. Obviously, in this stage, it is meaningless to speak of "determination" without further qualification; for, a certain area may be definitely "determined" while its constituent cells, at the same time, are not. Some stable order and localization has appeared in the germ, but it has not yet reached down to the ultimate elements.

4. Still *earlier* in development, we miss definiteness of trend even among larger cell masses; the parcellation of the germ into numerous dynamic centers, such as described in the preceding point, has not yet taken place. At the earliest stage accessible to experimental tests, only a few and very comprehensive fields are demonstrable.

Preformation and epigenesis in "regulative" forms.

As a matter of extrapolation, one could maintain further that every germ starts to develop from an initial state of *complete homogeneity*, possessing just one totipotent field. One could imagine that all later fields are derived from this primordial one by gradual emancipation. Theoretically, this view is incontrovertible, since we know that fields do disintegrate into sub-fields. But whether the process actually starts from a single unitary field, or whether there are several fields present in the egg to begin with, is a question of fact, not of theory; and the facts thus far revealed do not support the unitarian idea. In the unsegmented egg of the sea urchin, there are already present two autonomous fields in clear segregation — the animal and the vegetative fields (p. 294). In the amphibian germ at fertilization we find three: an animal (ectoblastic), a vegetative (entoblastic), and a marginal (mesoblastic) field (p. 351), each of which has special properties. These primordial fields are not totipotent, and, in fact, differ considerably in their capacity to produce complete individuals; the vegetative field of the sea urchin and the marginal field of the amphibian are much more nearly totipotent than the other fields. Nevertheless, the capacity for autonomous, even though incomplete, development of these different portions of the undivided egg, must be recognized, and this implies *plurality of initial fields;* in other words, the *pre-localization* of, at least, two field districts in the egg at the time when it first becomes accessible to our investigation. This is a very modest degree of *preformation*, but preformation it is.

After that, development bears a strictly *epigenetic* character, and *progressive determination* — the gradual fixation of the fate of each part in accordance with the field conditions to which it is exposed — becomes the ruling principle.

The universality of the principle of gradual determination.

Is *gradual determination*, however, the universal principle of development? If so, it would have to apply to *all* forms of animals, not just those we have here used to exemplify it; more particularly, it would have to embrace also those forms which earlier in this part (p. 234 ff.) were rated as true "mosaic" forms on account of their lack of regulative behavior. Our problem, therefore, is to harmonize the results obtained in the two groups of development, regulative and mosaic. This task is easier today than it may have seemed in earlier days of embryology.

On what facts has the distinction between regulative and mosaic types of development been based? Primarily, on the different behavior of germs damaged in early segmentation stages. Blastomeres were removed. If the rest of the germ gave rise to an essentially complete embryo, the egg was classified as a *regulative* egg; if development was defective, the egg was designated as *mosaic.* As one can readily see, *identical segmentation stages* were compared, as if the timing of segmentation steps could offer an absolute time standard of universal validity. This is the clue to the solution of our problem, because if we abandon the unwarranted assumption of segmentation being the clock by which to measure developmental progress, the apparent subdivision of animals into two distinct and antagonistic developmental types disappears.

"Regulative" and "mosaic" behavior — stations along a common course.

The facts are simply these: by the time cleavage sets in, the so-called mosaic egg has already incurred such a high degree of internal segregation and localization that the removal of a blastomere entails an appreciable defect. There is a time when an amphibian egg, too, can be found in precisely the same condition, namely, when it has reached the stage of organ primordia (p. 320). We know that the ablation of the field district of an eye or ear or limb entails the absence of these organs in the embryo (p. 321). But we also know that this is by no means a primordial condition and that, if the operation is performed at a somewhat earlier stage, the same amount of localized destruction fails to have a lasting effect on the organization of the embryo. If, thus, the mosaic state of the amphibian germ is clearly a secondary condition, why should the same not be true of the eggs of the so-called mosaic types? In other words, might it not be that they, too, start from a less rigidly determined state and become converted into mosaics only secondarily? If this is the case, the only crucial difference between "regulative" and "mosaic" eggs would lie in the fact that the latter have passed the "regulative" stage and become rigid mosaics before cleavage has even started, while the former do not attain this condition until well toward the end of cleavage or even later. This immediately raises the question as to whether eggs which behave as *mosaics* when mutilated *after the onset of cleavage*, might not be caught in a more "*regulative*" condition when tested in their *pre-segmentation* period.

Regulative performance of a "mosaic" egg.

The technical difficulties of a defect experiment on the undivided egg are great, because many eggs collapse when one tries to remove part of their substance prior to cellulation (see p. 238). Some eggs, however, survive the treatment; the sea urchin egg belongs in this group, as can be seen from example 45. Fortunately, there is also an egg of the mosaic type which is sufficiently resistant to permit the suggested operation, namely, the ascidian egg.

Example 80.

In example 25, the results of bisecting an ascidian egg meridionally in the two-cell stage or later were described. Each half develops the corresponding half of an embryo. When an egg is divided *before fertilization*, however, an entirely different result is obtained.[1] Both halves, the one containing the egg nucleus, as well as the non-nucleated (merogonic, p. 183) one, can develop after they have been fertilized. *And both halves can give rise to complete harmonious larvae of reduced size* (Fig. 87).

[1] *Dalcq*, 1938. Some regulative effects in the unfertilized ascidian egg bisected before fertilization have been recorded by *Dalcq* in 1932.

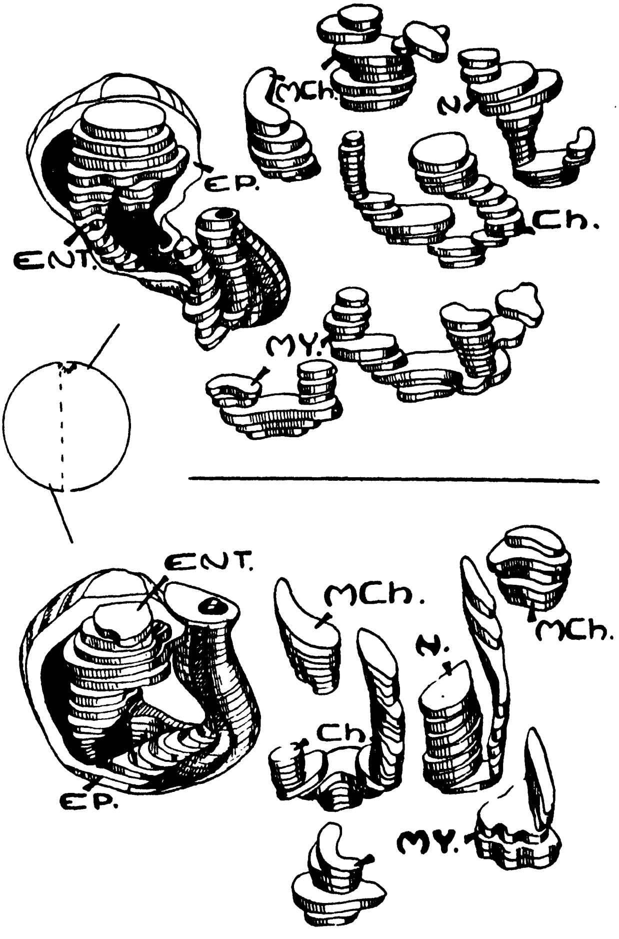

FIG. 87. Twinning in ascidians after meridional bisection of the unfertilized egg. (From *Dalcq*) The unfertilized egg was divided by a cut along the dotted line, leaving the egg nucleus in one half. Both halves were fertilized and developed into complete larvae. The pictures show reconstructions from sections of the larvae taken apart into the various organ systems. Each larva has a full set of organs, that is, median notochord (Ch), median neural cord (N), central entoderm (Ent), bilateral musculature (My) and bilateral mesenchyme (MCh). Ep, Epiblast (ectoderm).

One realizes, therefore, that even the ascidian egg, which usually is considered as a prototype of mosaic development, is by no means a mosaic in the sense that each partial phase of development is fixed and pre-localized in a definite parcel of the egg. The formation of a whole embryo from the reduced egg mass presupposes that each individual part of the egg has developed into something different from what it would have formed in normal undisturbed development. This proves conclusively, first, the *lack of detailed pre-localization in the virgin egg*, and, second, *the operation of organizing factors* of the same order as in the classical "regulative" eggs.

Further reconciliation between the mosaic and regulation concepts.

These experiments, indeed, provide the missing link between the "regulative" and "mosaic" types of development. They show that it is not only unwarranted but, in effect, contrary to the facts, to maintain that there is any such fundamental discrepancy between the two groups as the terms intimate. If we retain, for the time being, the terms "mosaic" and "regulative" types, we do this merely for convenient reference to the groups customarily so designated, without accepting the implications formerly attached to the distinction. In fact, the real demarcation between the two groups never appeared quite so sharp as one might infer from their rigid classification, and the more forms one examined, the more the distinction lost its sharpness. This may be briefly illustrated by an example.

Example 81.[1]

Annelids rank among the prototypes of mosaic development. A well-investigated annelid egg is that of the fresh water worm, *Tubifex*. The mature egg contains at its poles aggregations of a specific protoplasm. During the first two cleavage steps this plasm is so disposed that it becomes all apportioned to one of the blastomeres of the four-cell stage. This cell (so-called D-quadrant) has the prospective function of producing chiefly the *mesoderm streaks* of the embryo, while the other blastomeres give rise to ectoderm and most of the entoderm. When the D-quadrant is suppressed, the three remaining blastomeres continue to divide and produce some histological differentiations, but *do not give rise to an organized worm.* This corresponds roughly to the results obtained with other eggs of the annelid-mollusc group (examples 23, 24, p. 238) and seems to mark the egg of *Tubifex* as a strict adherent to the mosaic scheme. However, when the D-quadrant is raised *in isolation, it gives rise to a complete, proportionate,* although undersized, *embryo,* despite the fact that the other three fourths of the egg have been removed.

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[1] *Penners*, 1926; *v. Parseval*, 1922.

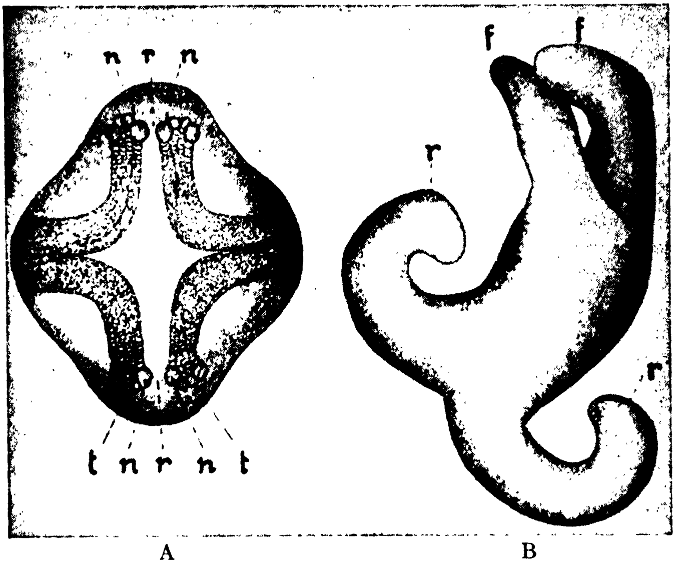

FIG. 88. Twinning in a worm. (After *Penners* from *Schleip*) Exposing the egg of *Tubifex* to high temperature (oxygen deficiency) causes a symmetrical repartition of polar plasms (see text) to both blastomeres of the first cleavage step. Each polar plasm later produces one pair of teloblasts; the embryo thus possesses two pairs instead of the usual one. The double mesoderm streaks developing from these two centers grow in opposite directions and deflect each other at right angles where they meet; this gives rise to a type of twin embryo known as *duplicitas cruciata.*

A, Double embryo.
B, The resulting double worm.
f, Head end. *r*, Tail end. *t*, Teloblast. *n*, Neuroblast.

Since these embryos possess all major parts, while ordinarily the derivatives of the D-quadrant would have formed only a circumscribed fraction of the embryonic equipment, "*regulation*" must have occurred. In other words, this egg could not have carried ultimate assignments in its constituent parts even as late as in the four-cell stage, much less at the beginning of cleavage. The superiority of the D-quadrant is attributable to the *polar plasms* in it. Twinning of these polar plasms is followed by the development of the equivalent of two whole embryos from a single egg (Fig. 88), and experiments in which the polar plasms were displaced by centrifugation have brought additional confirmation of their organizing role. At the same time, however, it has become clear that no further mosaic-like subdivision exists either within the polar plasms or within the other portions of the egg.

Thus, another "mosaic" egg is linked to the more patently "regulative" group, bridging what was once considered to be a natural gap. The demonstration of "mosaic" features in the prototypes of "regulative" development, the sea urchin (example 26) and the amphibian (p. 350), has helped to fill the gap. Even more illuminating is the discovery that closely related forms can exhibit almost all intergradations between the "mosaic" and the "regulative" extremes of the scale. This was found to be the case in *insects.*

Example 82.

Intergradations.

In example 76 we described the fact that some insect eggs can form whole embryos from egg fragments, which is a "regulative" performance. This same result, however, cannot be secured in all types of insects. The egg of a *Dipteron* (e.g., a fly), at the time when it is laid, is already so advanced in determination as to present itself in a real mosaic state. To test it, one kills a circumscribed area of the surface blastoderm by heat or radiation. In the *Neuroptera* (e.g., dragon-fly), as we have seen (example 76), this reduction of the germ does not preclude the development of a proportionate embryo. But in the

Diptera it always entails a permanent *localized defect.*[1] The same is true of *Coleoptera* (beetles, Fig. 89).

This proves that the various regions of the blastoderm of a fly or a beetle are in a different state than those of a dragonfly, if we compare them all at the time when the eggs are laid, that is, before cleavage has begun. At this moment, pre-segmentation development of different forms has obviously reached very unequal levels of progress. In the *Diptera* and *Coleoptera,* segregation has reached the point where organ-specific areas have become irreversibly localized, while in the *Neuroptera* such localization does not take place until much later. Other insect groups thus far studied — *Orthoptera, Hemiptera,* and *Hymenoptera* — take intermediate positions connecting the extremely "regulative" *Neuroptera* with the extremely "mosaic-like" *Diptera* in the indicated order.[2] That is to say, any later member of this list behaves in a more "mosaic-like" fashion than any of the preceding ones.

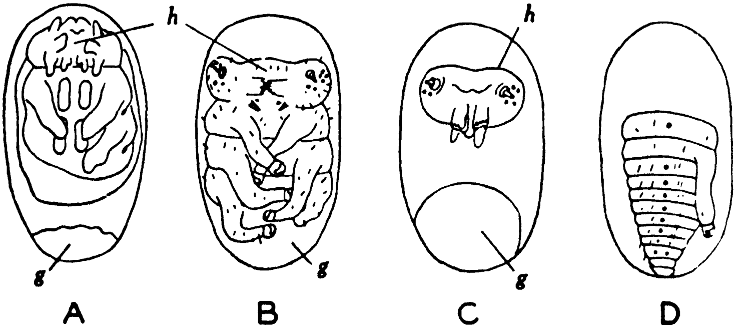

FIG. 89. Development of embryonic fragments after destruction of parts of the blastoderm in the beetle, *Leptinotarsa decemlineata.* (After *Hegner* from *Schleip*)

A, Anterior fragment (head and part of thorax).
B, Anterior fragment (head and thorax).
C, Head fragment.
D, Posterior fragment (abdomen).
h, Head. *g*, Destroyed region.

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[1] *Hegner*, 1911; *Reith*, 1926.

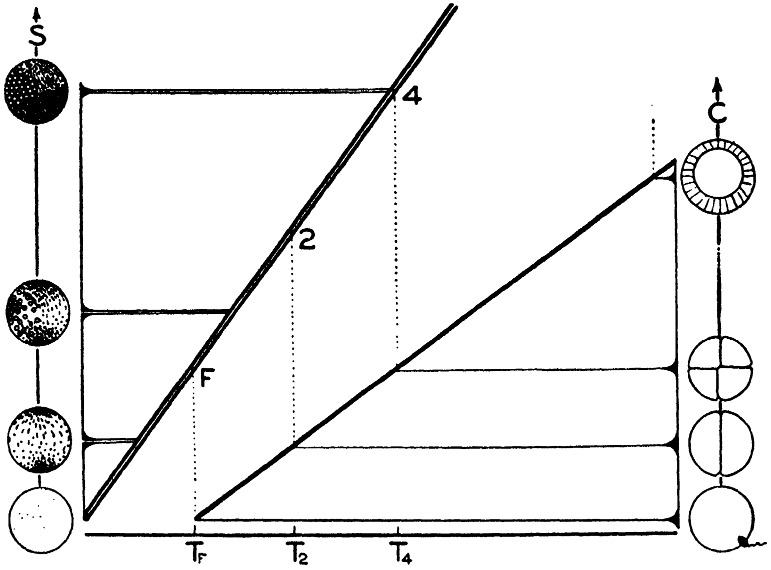
[2] *Seidel*, 1936.

Variations of developmental chronology link mosaic and regulative forms. There is, therefore, a solid basis for our contention that fundamentally development in *all* forms connotes *gradual determination,* rather than detailed pre-localization, although different groups evidently differ considerably regarding the speed with which localization occurs and the progress it has achieved at the onset of cleavage. Logically, therefore, we must date the beginning of development from the onset of inner segregation rather than from the moment of activation, fertilization, or first cellular division, which varies within very wide limits from one group of animals to another. Development of the egg begins when its parts begin to assume greater independence, determination, and localization than they had at the time when the egg was first formed. In most cases one may have to go back to the maturation or even pre-maturation period of the egg, in order to find the first traces of development as conceived in these terms. But, since there are no immediate visible criteria of the progress of segregation, it seems futile to attempt a precise, chronological identification of the moment at which it starts. After it has started, it proceeds at a rate distinctive for each species. There are fast developers, in which a high degree of emancipation of parts is reached comparatively soon, and slow developers, in which considerable time elapses before the erstwhile indeterminacy of parts has given way to more stable determination.

But there are still other variables to be considered in addition to the rate of segregation; these are the *time when segmentation begins,* and the *rate at which it proceeds.* On an earlier occasion (p. 223) we have explained that segmentation and differentiation are two discrete, collateral events, basically independent of each other, although normally tied into a cooperative association. Their independence, already verified experimentally, is reaffirmed by the fact that they vary independently among different species. That is, the time lines of segmentation and of differentiation are essentially independent curves whose points of intersection and slopes of progress vary from one form to another. The intersection, marking the onset of segmentation, lies very early in development in forms in which *fertilization occurs early.* In this case the first blastomeres and their early derivatives appear at a time when localization in the egg, if indeed initiated at all, is still very vague. Therefore, isolated blastomeres can still be totipotent, and segmentation will be far along by the time the developmental segregation has transformed the egg into a mosaic of localized fields. Such eggs are classed as "*regulative*" (Fig. 90, bottom). In other forms, cellulation may begin very *late* relative to the onset of development, and by the time the first furrow cuts through the egg, the emancipation of parts may have reached such an advanced state that the removal of a blastomere in the two-cell stage eliminates a definitely segregated portion of the future organism, while the remaining portion is too firmly consolidated to repair the loss. This is then usually regarded as a "*mosaic*" egg (Fig. 90, top).

Summary: Fundamentally, development is the same in all forms.

To sum up, eggs with *rapid differentiation* and *comparatively late start of cleavage* compose the so-called "*mosaic*" group, because even at the earliest accessible cleavage stages the egg is already past the state in which parts could be freely substituted for one another. Conversely, eggs which are *slow of development and in which cleavage comes in relatively early*, form the so-called "*regulative*" type. This situation is diagrammatically represented in Figure 90. One must keep firmly in mind, however, that there is a *continuous gradation from one extreme to the other.* Furthermore, even in the most extreme "mosaic" types the original mosaic is crude, and *progressive determination of details*, attended by specific interaction of parts continues (compare example 24). On the other hand, even the eggs of the most extremely "regulative" type display at the time when cleavage begins already some degree of pre-localization, as evidenced by the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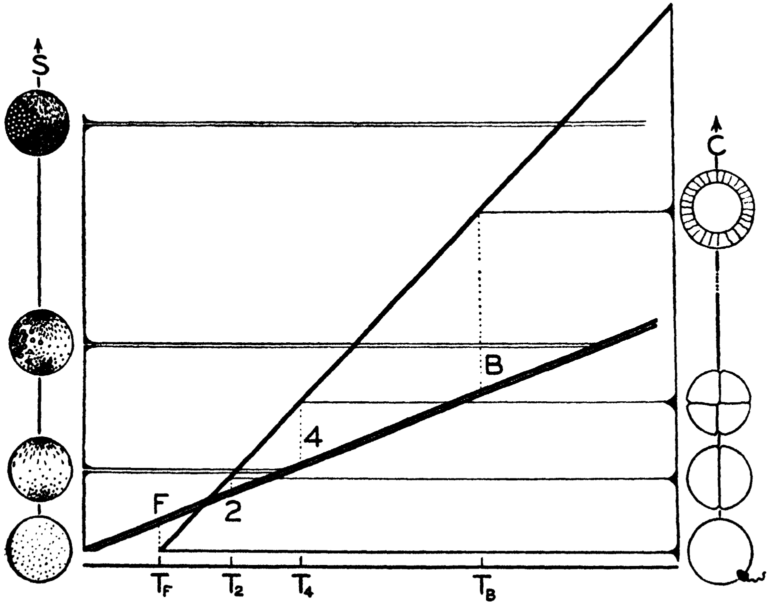
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presence of an animal and vegetative field in the sea urchin and of the ectoblastic, marginal, and entoblastic fields in amphibians. We conclude, therefore, that *the so-called "mosaic" and the so-called "regulative" types of development are merely chronological variations of one and the same fundamental aspect of development — gradual segregation and progressive* [1] *determination.*

THE COMPOSITE CHARACTER OF DETERMINATION

Determination by Degrees

The multiplicity of determinating agents.

Intent on documenting the operation of this principle of gradual determination, we have omitted thus far to lay due stress on the *heterogeneity and multiplicity of determinative agents.* When we speak of an area of the germ as becoming "determined" for some local formation, then of continued "determination" defining the fate of smaller groups of cells, until eventually the histological and functional character of each individual cell has become "determined," this cannot fail to give the impression that all this is effected by the operation of a single controlling agent. Yet, this impression is absolutely misleading. There is no single agent which could be considered the master clue to the understanding of all developmental phenomena.

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[1] In speaking of "progressive" determination, we have in mind progress in time, not in space.

FIG. 90. "Mosaic" and "regulative" development — a result of independent chronological variation of segregation and cleavage. In both the upper and lower diagrams, two independent ordinate systems have been plotted against the same time abscissa. Left ordinate (S; double lines): Progress of inner segregation (emancipation of parts) within the germ, attended by the emergence of localized fields. Right ordinate (C; single lines): Progress of cleavage up to the blastula stage.

Top: Relatively fast segregation, late fertilization, slow cleavage. At the moment of fertilization (T_F), segregation has already set in. At the 2-cell stage (T_2) and 4-cell stage (T_4) segregation has reached an advanced level. No complete "regulation" can, therefore, be obtained from isolated blastomeres. Extreme "mosaic" type.

Bottom: Relatively slow segregation, early fertilization, fast cleavage. At the time of fertilization (T_F), and even at the beginning of the 2-cell stage (T_2), segregation has as yet made little progress. Even at the blastula stage (T_B), localization has not yet reached the level which the "mosaic" germ above passes between F and 2, i.e., prior to the 2-cell stage. Consequently, complete "regulation" of certain egg fragments is possible up to the blastula stage. Extreme "regulative" type.

In this connection, it is well to remember the distinction between principles and agents (p. 106) and the fact that uniformity of principles does not imply uniformity of agents. In Part One the diversity of developmental characters has been illustrated. The list contains manifold and disparate characters. The production of a specific secretory apparatus in certain cells — a typical differentiation — cannot be treated on the same basis as the arrangement of these cells into an epithelial layer. The differentiation of neurofibrils and the sending out of an axone from a nerve cell are phenomena of a wholly different order from those effecting the transmissive connection between this axone and a muscle fiber. Some features pertain to the individual cells as discrete elements; while others pertain to their grouping and relationships; some pertain to active performances, while still others refer to purely passive transformations which the cells suffer through mutual contacts. "Determination" of a district to become eye is something entirely different from the "determination" of an individual cell to become a nerve cell. The two events are on a different plane, and it does not seem rational to pretend that there could be any single agent so versatile as to manage them both. If development is a *composite* product, so is "determination." Progressive determination may be a *principle* common to all development; but the *agents* through which it operates are great in number.

Determination of morphogenetic movements.

Let us illustrate this fact by a few selected examples. Some have already been mentioned. We have reported (p. 310) that there are *pre-determined* characters. Next, there is determination of *cleavage* conditions; of *cell movements;* of *growth stimulations* or *inhibitions;* of specific *cytological* and *histological differentiations;* determination of *mutual adjustments,* such as between a nerve and its end organ, or between the two parts of a joint; determination of the amount, distribution, and termination of *vascular* and *nerve* supply; and indeed determination of all those characters which were specified in Part One, and many more. Determination of cleavage pattern was discussed in example 45. We shall now briefly consider *determination of movements.*

Example 83.

The extensive shifts of germinal material which get under way at the beginning of gastrulation and end up with the establishment of the germ layers (p. 341) follow essentially three courses (in amphibians): (1) *invagination* of the marginal zone around the rim of the blastopore; (2) *expansion* of the presumptive ectoderm; (3) *sinking in* of the vegetative material into the interior to form the entoderm. To an observer the whole gastrulation process gives the impression of a uniform act, and he will ask which one of the three movements is its motor. Experimental tests, however, have shown that each component has its own motor and can proceed independently of the others. Thus, *marginal fragments* of the blastula, if transplanted into an otherwise inactive region of a host germ *continue to invaginate* and may even drag neighboring materials of the host beneath the surface [1] (see also example 61). A fragment of transplanted *presumptive ectoderm* in the same location behaves quite differently. It stays in the surface and tends to expand tremendously. *Vegetative* material, finally, when grafted into the surface of another germ, invariably pushes into the interior and submerges.[1] Even in complete isolation (tissue culture), ectodermal epithelium of the blastula continues to expand.[2] In other words, *each fragment has proved capable of automatically producing its proper gastrulation movement* without help from the others. If the same experiments are repeated with fragments taken from a germ past the stage of gastrulation, the grafts no longer show any tendency to stretch, roll in, or sink in, as the younger ones did. Thus, the urge to move in certain directions is only a temporary acquisition of the materials concerned.

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[1] *Mangold,* 1923.

[2] See Figure 74.

These results show that, contrary to what one might have expected, gastrulation is *a composite act*, and that the roles to be performed in it have been distributed— "determined" — well in advance of the actual performance; namely, prior to the blastula stage. On the other hand, evidence reported above, especially in examples 48 and 49, proves that this determination of gastrulation movements cannot possibly prejudice the further developmental course of grafted fragments. Even after gastrulation has started, such fragments can still be interchanged and made to develop in accordance with their new locality. Thus, we see that *the factors determining the shift of germinal materials into their definite places are not identical with the factors determining the further transformations of these materials.*

Gradual progress of determination, illustrated on the heart.

The subsequent determination of shape and structure is again a complex phenomenon in which numerous independent processes are coöperatively engaged; some of these operate concurrently, others in succession. Experimental measures are required in order to decompose the composite history of, for instance, an organ into its constituent "determinative" phases. A few examples will help to clarify the point.

Example 84.

According to example 29, a circumscribed district of the ventral mesoderm of an amphibian germ which is past a certain critical stage can self-differentiate into a heart. But when one says "heart," one does not usually consider the diverse features the term implies. To mention only a few: a heart is a hollow tube, muscular, twisted, subdivided into chambers, pulsating. The capacities to develop these features are acquired neither abruptly nor simultaneously. They come in one by one. The first to be laid down is the capacity to produce *rhythmically beating muscle.* When presumptive cardiac mesoderm of a urodele neurula is explanted, it merely differentiates into an *unorganized mass of muscle cells* which soon begin to *pulsate*, but do not otherwise resemble a heart.[1] *Histogenesis, therefore, is determined prior to morphogenesis.* Explanted at a slightly later stage, the mass develops into a *pulsating tube;* although there is still not further subdivision, some trace of morphological development toward heart is, at least, indicated. Explants from still later stages develop into *pulsating tubes subdivided by constrictions* into four sections, representing sinus, auricle, ventricle, and bulbus. Only explants or transplants from still more advanced embryos can self-differentiate into a structure which actually resembles the normal end stage of a heart.[2] Even then, however, lacking connections with a functioning circulatory system, the details of internal organization, particularly the amount and arrangement of muscular tissue, differ considerably from normal.[3]

According to these experiments, the organ which eventually appears as heart has *acquired its principal functional and structural properties in a series of events and at different times:* first, the capacity to pulsate; second, the capacity to form a tube; third, subdivision of this tube; fourth, twisting of the tube into the typical S-shaped condition. And this list contains only the main stations on the way. More detailed studies would undoubtedly reveal a still greater variety.

Example 85.

Gradual progress of determination in the lens.

In example 56 the ability of an eye vesicle to provoke *lens formation* in the overlying skin has been demonstrated. Further experiments, however, have shown that this initiation of a lens-producing process does not at the same time convey to the responding tissue additional instructions concerning the ultimate size and orientation of its product. It is not until much later that these characters become fixed. After the lens vesicle has become pinched off from the skin, it differentiates into two very unequal portions: the mesial wall, that is, the one facing the retina, forms the solid mass of lens fibers, while the lateral wall remains a flat, indifferent epithelium (cf. Fig. 11). However, the *position of the fibrous portion is not determined at the same time as the capacity of the rudiment to differentiate fibers.*[1] For, a change in the orientation of the lens rudiment, even after the latter has become firmly set on giving rise to a lens, and to no other structure, can still produce a shift of the fiber-forming zone. Inside the eye, direct exposure to the retina seems to force the cells so exposed into the fibrous course. But exposure to other sensory epithelia, e.g., the sensory cells of an ear vesicle or a nose placode, is equally effective.[2] Wherever there is any such epithelium in the immediate vicinity of a developing lens, the adjacent part of the vesicle transforms into the fiber mass; and if a lens is placed in between two sensory epithelia, so that it is in contact with both, a double center of fiber formation can arise in it (Fig. 91).

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[1] *Goerttler*, 1928.
[2] *Ekman*, 1924; 1927; 1929.
[3] *Stöhr*, 1925.

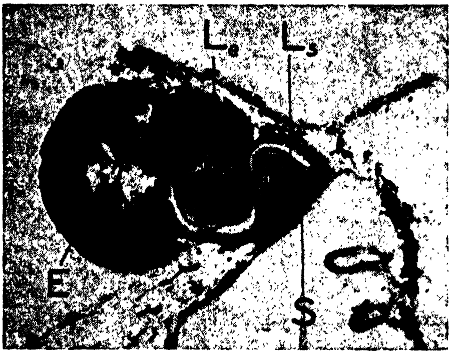

FIG. 91. Lens with two fiber centers. (From *Dragomirow*, 1929) Eye cup (E) transplanted into the vicinity of the ear has induced lens which has developed one body of fibers (L_e) in contact with the retina and another body of fibers (L_s) in contact with the sensory epithelium (S) of the labyrinth. × 108.

While we are ignorant of the exact nature of the factor which localizes lens fiber differentiation, the experiments indicate that it consists of some action common to all sensory epithelia. It is clear, furthermore, that this factor becomes operative only after the general quality of the lens, as such, has been determined. After the position of the lens has been fixed; after its shape, polarity, and orientation have been determined; after the histological differentiation of its cells has gotten under way, there still remains one variable to be considered, namely, *size.*

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[1] *Woerdeman*, 1934.

[2] *Dragomirow*, 1929.

Determination of Size

Growth and size.

For some comments on the problem of size, we refer to Part One. Size is a product of growth; hence, size determining factors are identical with growth determining and growth limiting factors. *Size is merely a measure of the cumulative effect of growth* attained up to the time of measurement. A certain amount of material increases at a certain rate over a certain length of time: the result is a certain size. Size, therefore, has a complex history, and so, likewise, has the determination of size. The problem of determination of size must, consequently, be decomposed into as many separate issues as there are conditions affecting growth. The major factors are: original mass of material, rate of proliferation, proportion of proliferating to non-proliferating cells, changes in cell size, production of inter-cellular ground substance, rate of destruction, stimulating or inhibiting factors in the environment, and the period over which growth continues.

Speaking of determination of size, one must be explicit about the phase of development one contemplates: embryonic development, larval development, adult form — all have definite size limits, which are not necessarily interdependent.

The role of initial mass.

With this qualification in mind, we can now turn to a closer inspection of the factors of size. The first factor calling for our attention is the *initial amount of living material* present in the developing system. Example 46 has furnished evidence that the initial mass does have a definite influence on the size of the organism at the end of embryogenesis. Larvae developing from egg fragments, although harmoniously organized, are definitely undersized. To avoid repetitions, we refer to the previous discussion of this matter on page 301. Another example will show that the conclusions reached there hold not only for the organism as a whole but also for its constituent parts.

Example 86.[1]

> When the micromeres of a sea urchin egg are isolated in the sixteen-cell stage (Fig. 43) and raised in sea water, they divide until each has produced approximately one dozen cells, and then stop. The final number of descendant cells thus produced *corresponds very closely to the normal number of skeletoblasts* which form from the micromeres in an intact larva (cca. 50; see p. 258).

According to this observation, there is apparently a *definite lower limit* beyond which cell size does not go. Each division reduces the size of the individual cells. Segmentation continues so long as the cells are still above the critical size minimum; after that further division is automatically checked. To this extent *the size of an organ at the close of the embryonic period may bear a definite relation to the amount of material assigned for its formation earlier in development.*[2] This relation cannot be generalized, however. As one can readily understand, it holds true only for species whose embryos cannot draw upon extrinsic nutrient materials and in which, therefore, no real growth, in the sense of augmentation of living substance, can take place during embryogeny (see p. 78). The more marked, however, the separation of an egg into an embryo-forming and a yolk-storing portion becomes, the more actual growth occurs during embryogeny, the embryonic portion increasing at the expense of the extra-embryonic materials. In these forms, therefore, the size of an organ at the end of the embryonic period depends not only upon the amount of material originally allocated to its formation, but likewise on its subsequent embryonic growth rate (see p. 37), the supply of nutriment, and the length of the embryonic period. If the final size of the progeny of the micromeres in example 86 was limited, this was merely due to the fact that the cells could not grow.

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[1] *Hörstadius*, 1935.

[2] The definite ratio between amount of germinal material and cell numbers is particularly striking in forms of the strictly determinative cleavage types in which organs are often composed of a fixed number of cells; experimental reduction of the size of the germ entails proportionate reduction of the number of cells.

Unlimited growth capacity of embryonic cells.

Embryonic cells with an opportunity to grow, however, are capable of producing practically unlimited offspring. This is conclusive proof that the size of an organ is not controlled by a definitely limited growth capacity of each one of its original cells. The culture of embryonic tissues brings out this fact very strikingly.

Example 87.

When a heart fragment of a chick embryo is explanted in tissue culture, it contains a definite number of cells which would, in the normal course of events, have produced a limited portion of the heart muscle. In *tissue culture*, however, where they are provided with an overabundant food supply, *these cells continue to grow and divide*, as it seems, *indefinitely*. Each cell grows and, after reaching its critical size, divides; the daughter cells grow up again and divide; then, their daughter cells repeat the performance; theoretically this could go on forever. In this manner, embryonic mesenchyme cells and myoblasts have been kept in incessant proliferation and growth over several decades without any perceptible exhaustion.[1] They behave, in this respect, much as a colony of amply fed protozoa. It has been calculated that this unrestricted growth can, within a short period, accrue to a mass of tissue which exceeds by a multiple the size of even the hugest animal.

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[1] *R. Parker*, 1938.

Since, according to these observations, the size of the progeny of a given embryonic cell is not intrinsically fixed, the question arises as to what factors limit growth in the organism, as against growth in tissue culture. Why do not cells in the organism proliferate indefinitely? The answer can be found in the fact that in the organism, *progressive differentiation and continual destruction steadily drain the proliferative sources.*

Growth checked by differentiation and destruction.

As we have mentioned on an earlier occasion, there seems to be a fundamental antagonism between proliferation and differentiation (pp. 85, 134). Cells kept in continual mitotic activity do not easily differentiate and, conversely, cells with highly specialized differentiations are not apt to divide. The reason for this antagonism has already been outlined. As differentiation transforms protoplasmic substance into specialized structures, it evidently reduces the plasticity of the cell content and encroaches upon space and material prerequisite for the building of the mitotic apparatus. A differentiating cell, therefore, gradually loses its capacity to divide, and *factors promoting differentiation automatically reduce proliferation.*

The second manner of elimination of cells is by *destruction.* Destruction of existing cells frequently takes place concurrently with the production of new cells and is particularly conspicuous in blood, skin, and glands. Blood cells disintegrate or are ingested by phagocytes; epidermis cells are shed after becoming cornified; and some gland cells explode in the act of secretion. These tissues, therefore, reach their final dimensions when production and destruction are in balance. As we have outlined in Part One, the fluctuating character of this stationary equilibrium brings us into the borderline field of physiological function proper.

One realizes, therefore, that the size of an organ is controlled both by the rate of production of new cells and the rate at which they are withdrawn from proliferation by differentiation and destruction. The rate of differentiation is controlled by the local conditions (field factors), to which the cells become subjected. This is a second way in which field factors affect final size: first, they are responsible for the initial amount of cells allotted to an organ rudiment (p. 317), and second, they control the type of differentiation, thereby regulating the decline of multiplication. *Growth continues so long as multiplicative activity remains in excess of the draining of the proliferating sources by differentiation and destruction.*

Inherent growth potentials of the species.

Like morphogenesis, the production of a definite size depends on a *multitude* of agents, partly intrinsic to the cells, partly residing in the surrounding organism, partly provided by the environment. In a sense, the relation between the individual cell and the organism, or the "inner milieu," is the same as that between the organism as a whole and its environment, the "external milieu." Consequently, if the growth rate of an organism is the resultant of an intrinsic growth potential and the growth circumstantials of the outer milieu (p. 22), the growth rate of a cell can likewise be decomposed into a *cellular growth potential*, setting the fundamental pace of cell growth, and the *circumstantials*, which are furnished by the rest of the organism and control the realization of growth. The problem of the factors determining growth rates, thus, consists of two parts, one pertaining to the potentials, and the other to the circumstantials. Both potentials and circumstantials change as development goes on. The general picture is as follows. (1) The fertilized egg possesses a constitutional growth potential as an inherited endowment. (2) The various cell strains descendant from the egg develop differences of growth potential as a corollary of their local differentiations. (3) Differentiation of the germ, furthermore, entails local changes of the growth circumstantials.

A few remarks may serve to qualify these statements.

Evidence that cells possess an *inherent growth potential specific for the species* from which they have descended, has already been recounted above, page 311. Undifferentiated cells taken from a germ of species *A* and transplanted into a germ of species *B* conform with the formative tendencies of their new location, but do so at a rate characteristic of the *donor* species *A*. Since these effects were recorded in comparatively early stages of development, one could argue that the transplanted cells, while unquestionably persevering for some time in their original rate, eventually might have become adapted to the growth rate of the host, had more time been allowed for the experiments. Later experiments, however, in which the grafts were observed over prolonged periods, have removed this objection. We now possess convincing proof that *the discordance of growth rates between tissues of different species never subsides* and, therefore, must be ascribed to differences inherent to the growing material.

Example 88.[1]

Heteroplastic grafts as tests of inherent growth potentials.

Limb development in different species of the urodele amphibian, *Amblystoma*, follows different time courses. In *Amblystoma punctatum*, for instance, the fore limbs appear relatively early in development and are functional and almost fully formed, including digits, by the time the larva reaches the swimming stage. In *A. tigrinum*, on the other hand, limb development is considerably delayed, and at a stage generally comparable to that just described for *A. punctatum*, the limbs are but inconspicuous nodules. Once they have started to grow, however, they proceed at a much faster rate than the *punctatum* limbs and soon overtake them. In order to test whether these specific time characteristics are expressions of immanent growth potentials of the limb-forming cells or of growth controlling influences of the body, reciprocal transplantations of limb buds were performed between embryos of the two species at a stage prior to

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[1] *Harrison*, 1935a.

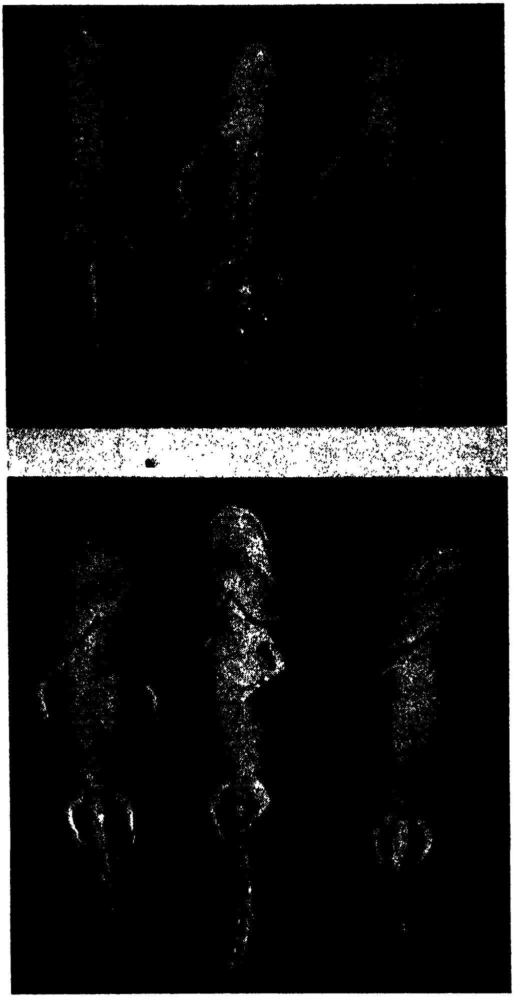

FIG. 92. Growth of limbs exchanged between species of different growth potentials. (From *Harrison*) All operations performed on right (in the photograph, left) fore limb.

Top row: *Amblystoma punctatum.*

Bottom row: *Amblystoma tigrinum.*

In the first animal of each row only the ectoderm of the limb bud had been replaced by ectoderm of the other species. These limbs have developed to *host* size, showing no effect of the grafted component.

Animals in the middle of each row had received whole limb buds (ectoderm plus mesoderm) from the other species. These limbs have developed to *donor* size.

In the last animals of each row only the mesoderm of the limb buds had been exchanged. Development has proceeded at the rate of the *donor.*

Result: The growth potential of the limb is an intrinsic property of the limb mesoderm.

the actual outgrowth of the limbs. The transplanted buds developed into functional limbs, but in so doing, *followed unmistakably the time schedule of the donor species.* The *punctatum* limb on the *tigrinum* host started its development much earlier, but grew more slowly and remained much smaller than the host's own delayed control limb. Conversely, a *tigrinum* limb on a *punctatum* body was late in starting, but, once on its way, developed with much greater rapidity and to conspicuously larger dimensions than the host limb [1] (Fig. 92).

Experiments of this type have been repeated with other organs, for instance, gills,[2] heart,[3] ear,[4] spinal cord,[5] and eye,[6] (Fig. 93), yielding more or less concordant results. Taken in conjunction, they establish firmly the existence of an *intrinsic and constitutional growth potential inherent to all cells of a given species and controlling their basic growth rate.* We understand now that the properties for which a species rates as either a fast grower or slow grower (p. 425) are not primarily the results of growth promoting or growth depressing agents circulating in the body, but rather are they constitutional properties of the protoplasm of that species, passed on to all cells. The protoplasm of different species seems to differ with regard to the avidity and facility with which it seizes, selects, and assimilates suitable nutriment, thereby increasing its own substance. This accounts for the observed growth differential between cells of different origin under otherwise identical circumstances. In the above example, for instance, the two limbs belonging to different species — one to the donor, the other to the host — develop in a common body. Both have access to the same supply of food and of other growth controlling stuffs. If, nevertheless, one grows faster than the other, this must be because its cells are better in seizing and utilizing these substances.

We emphasize species differences simply because these are conspicuous enough to permit a clear cut demonstration

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[1] A *punctatum* limb on a *tigrinum* body may even remain somewhat smaller than the control limb of the *punctatum* donor, while a tigrinum limb on a *punctatum* body may overshoot the dimensions of the donor control. This apparent exaggeration of the innate growth potential is, however, a secondary effect which can be corrected by controlled feeding (*Twitty* and *Schwind*, 1931).

[2] *Harrison*, 1929b; *Rotmann*, 1931.

[3] *Copenhaver*, 1927, 1933.

[4] *Richardson*, 1932.

[5] *Detwiler*, 1931, 1932; internal adjustments, however, cloud the original growth potentials.

[6] *Harrison*, 1929a; *Twitty* and *Elliott*, 1934.

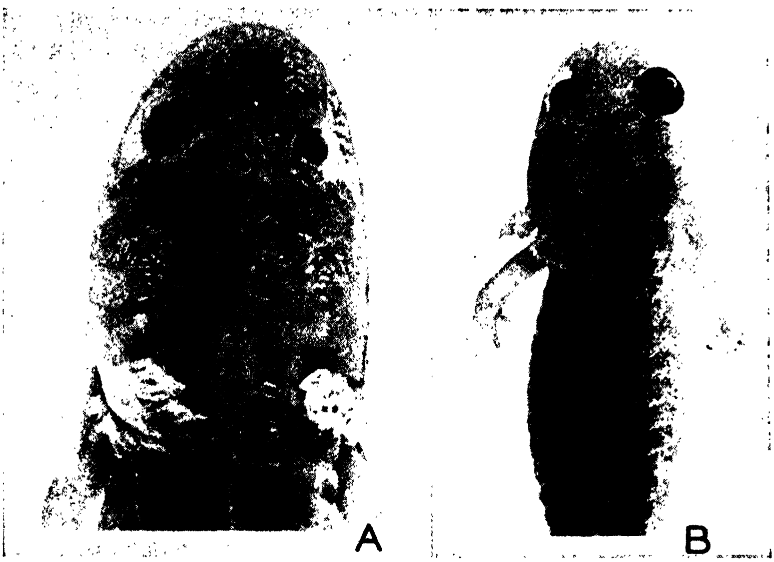

FIG. 93. Reciprocal grafts of embryonic eyes between two species of *Amblystoma*. (From *Twitty and Elliott*)

A, *A. tigrinum* with right eye from *A. punctatum*.
B, *A. punctatum* with right eye from *A. tigrinum*.

Both animals were of identical embryonic stage at the time of the eye exchange. A has grown much faster than B. The grafted eyes have assumed *donor* size (after initial deviations). Both animals were maximally fed. (The eyes of the preserved specimens have been exposed in full size by the removal of the surrounding skin.) × 3.3.

of the *inherited character of growth potentials.* Similar constitutional differentials exist, however, on a smaller scale, between races, families, and individuals. Every individual germ, according to its genetic constitution, possesses an innate growth potential which remains the fundamental standard of all further growth.

The development of differential growth potentials.

Given a fundamental growth potential of all descendant cells of a germ, the question arises as to how those local differences of growth rates arise which are responsible for the *differential growth* of the organism (p. 36). Gills, limbs, lungs, kidney tubules, and a host of other structures expand much more rapidly than the rest of the body. Is this the result of locally different growth circumstantials which make cells of uniform growth potential grow faster in one place than in another, or does the original growth potential gradually change as the cells differentiate? More concretely, does a limb, for instance, grow faster than the body wall because some stimulating effect of the limb base is constantly firing the cells to increased activity, or do limb cells, as they become limb cells, acquire an intrinsically greater growth efficiency? The fact that a self-differentiating transplant in a strange location can attain nearly normal size and proportions seems to support the latter alternative. The following observations confirm this view.

Example 89.[1]

> Mesenchyme strains were recovered from different locations of a chick embryo and were reared in tissue culture under practically identical conditions. The explanted fragments, embedded in a clot of blood plasma and provided with measured quantities of nutrient medium, proliferated extensively during the first days after explantation. *The amount of this outgrowth, however, varied distinctly for the various strains used.* By repeated trimming and transferring of the cultures to fresh media, the original fragment was gradually eliminated so that, eventually, the cultures consisted purely of cells born during the period of cultivation. Still, the differences between the growth rates of the different strains persisted (Fig. 94). Inasmuch as any residual effect of the original organismal environment of the cells would have become diluted to the vanishing point in the course of successive transfers, the observed differences in growth rate must be ascribed to the *intrinsically* different growth potentials of the different strains.

These results suggest that part, at least, of the differential growth of the organism is based on *acquired modifications of the intrinsic growth potential* of the cells concerned. Since, however, the number of cells accumulating around an explanted fragment in tissue culture is not a true measure of cell multiplication, but depends on the degree of cell migration as well, these observations are in need of more crucial tests.

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[1] *R. C. Parker*, 1929.

Naturally, the alignment into which the cells are brought by morphogenetic field effects is a major factor in producing local inequalities of growth. It must make a great deal of difference to the growth rate of an organ whether dividing cells are uniformly scattered throughout the mass or whether, owing to unfavorable conditions in the interior, division can only proceed on the surface, as, for instance, in the numerous examples of epithelial organs where the proliferating cells are confined to restricted zones (pp. 50, 86). The growth rate of a cell, therefore, depends not only upon its inherited and ontogenetically modified growth potential, but, likewise, on its *position in the field* and the *mechanical, chemical, electrical, etc., conditions resulting therefrom.* Moreover, field action, by directing differentiation, also affects that increase

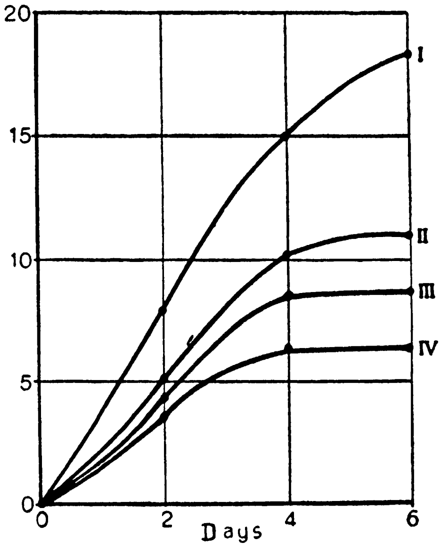

FIG. 94. Differential behavior of different strains of mesenchyme after prolonged cultivation in vitro. (From *R. C. Parker*, 1929) Strains of embryonic mesenchyme, recovered from bone (I), cartilage (II), skeletal muscle (III) and heart (IV), were cultivated under identical conditions for several weeks and then tested for their capacity of migration and growth in a medium practically devoid of nutriment. The graph represents the "residual growth energy" of the four strains as manifested in the test medium. Abscissae: Time after transfer into test medium. Ordinates: Amount of outgrowth (i.e., area covered by the outgrowing cells; G in Fig. 22).

in bulk which is a direct outcome of differentiation: the secretion, deposition and accumulation of intercellular ground substances such as are observed in the connective tissue, cartilage, bone, etc.

The growth circumstantials inside the organism.

If the determination of growth potentials is, in itself, a composite process, further complication arises from the diversity of conditions under which growth potentials become actual growth. As circumstances influencing the realization of growth of the organism as a whole, we have mentioned (p. 19 f.) temperature, composition of the milieu, food, and social factors. The differential effect on growth of a change in the temperature level has been illustrated (p. 45, Fig. 8): a general speeding up of growth by a rise of the temperature level benefits the fast growing parts to a greater extent than the more slowly growing ones. This can serve as an example of how a general change of a growth circumstantial tends to exaggerate small initial differences.

The *food supply* inside the germ is usually freely accessible to all parts, either by diffusion or, in larger embryos, by early vascular circulation, so that this factor, too, cancels out as a potential source of differential growth. But, as we have said above, the several parts of a germ share the common supply of food stuffs according to their relative capacity to absorb them, their "*partition coefficient*," [1] as it has been called. These partition coefficients are expressions of the intrinsic growth potentials. They are so balanced in normal development that no structure can monopolize the available food supply to the detriment of the others. This is different when a serious shortage of food supply or building material arises. Then the competition and struggle among the parts for the limited reserves often lead to fragmentary formations in which only the strongest parts survive at the expense of the weaker ones. Not uncommonly, for instance, one of the two partners of a pair of fused twin embryos, such as sometimes occur in fishes, happens to be more vigorous than the other; the stronger one, then, by withdrawing all the available supply for its own use, starves the weaker one, with the result that the latter becomes gradually reduced and absorbed.[1]

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[1] *Robb*, 1929.

The *social factors* affecting the growth of individuals (p. 20) find a counterpart within the organism in the form of mutual influences among the growing parts. Most of these actions are commonplace and need no further discussion. One of the most obvious is spatial limitation imposed upon any growing part by the mechanical configuration of its surroundings.

Greater interest, however, is offered by the *chemical* and *physico-chemical composition of the internal milieu* in the embryo which, in a way, is the resultant of chemical actions of all parts. Just as the composition of the external milieu affects the growth of the whole organism, so the internal milieu has its effects on the growth rate of the individual parts. The ion content, pH, viscosity, dispersity, electric charge, of the body fluids, as well as their content of specific growth promoting substances and hormones (p. 142) are all factors that affect growth rates.

Mutual adjustments of growing organs.

Furthermore, *growth adjusting mechanisms* operate between parts which are neither crudely mechanical nor of the type of diffuse milieu action just discussed. They are local and contribute to the coördination of growth in neighboring structures. This brings us back to our starting point — the determination of the size of the lens. Although of different origin, the lens usually matches the size of the eye cup perfectly. How is this correspondence, then, achieved if it is not sheer coincidence?

The first hint comes from observations on embryos with experimentally reduced eye vesicles. It can be seen from Figure 95 that lenses induced by undersized eye cups are correspondingly undersized, so that one may conclude that the initial material reclaimed for lens formation is already in some definite proportion to the actual size of the eye cup. This early and unilateral size adjustment is later supplemented by mutual growth interactions between lens and eye whose operation is strikingly demonstrated by the heteroplastic grafting experiments described in the following example.

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[1] *Stockard*, 1921.

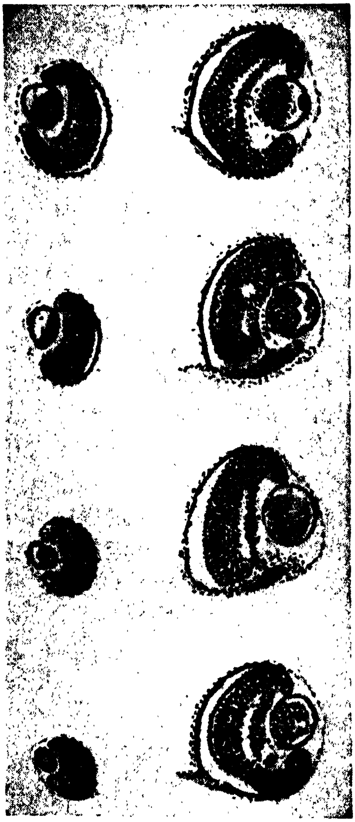

FIG. 95. Size relations between eye and lens. (From *Spemann*) The picture shows four instances of experimental reduction of the left embryonic eye cup of the anuran, *Bombinator pachypus*, with the right undisturbed eye serving as control. It can be seen that the lenses whose material was not directly affected by the operation have assumed correspondingly reduced sizes.

Example 90.

Example 88 has shown that in the growth response of limbs reciprocally transplanted between two species of different growth characteristics, the indigenous growth potential of the limb prevails. The same is true of the gills, the heart, and some other structures. Some composite organs, however, do not behave in precisely the same way. Let us consider, for instance, the system, eye plus lens. If the early eye vesicles with the attached lens rudiments are exchanged between *Amblystoma tigrinum* and *A. punctatum,* the results are essentially the same as in the case of the exchange of limbs.[1] The *tigrinum* eye, which has the higher growth potential, reaches the large size of a normal *tigrinum* eye although the *punctatum* host grows more slowly; while the *punctatum* eye in the *tigrinum* body remains as small as it usually is in its own body (Fig. 93). If, however, the two components of the eyes are separated and an optic vesicle of one species is combined with the lens of the other species, a markedly different result is obtained. If each component grew again to its customary size, regardless of the other, the result would be, in one combination, a giant *tigrinum* eye with a tiny *punctatum* lens, and, in the reciprocal combination, a small *punctatum* eye with a huge *tigrinum* lens. In reality, however, a sort of compromise solution is effected and the potential *disproportion between eye and lens is smoothed out:* the growth of the *tigrinum* component is held back while that of the *punctatum* component is accelerated so that, in the end, fairly *harmonious* eyes result, whose lens matches the cup and whose size is intermediate between that of normal *tigrinum* and *punctatum* eyes.[2]

Evidently, eye cup and lens have mutually adjusted their growth rates to each other. A similar reciprocal size regulation operates in adapting the two parts of a joint (p. 433). This is the type of correlative growth in which the growth of two discrete parts is controlled by mutual interdependence. But this is not the only type known. There are also growth controlling actions which are strictly *unilateral.* It has been found, for instance, that a huge tigrinum eye in a small punctatum body causes many of the accessory structures of the vicinity, such as muscles and cartilage, which are formed by host tissues of intrinsically lower growth potential, to grow to such excessive dimensions that they match the graft.[1] Unlike the lens (example 90), these structures do not on their part depress the growth of the graft. Similarly, the heteroplastic combination of limb mesoderm of one species with skin of another species, proves that the growth rate is determined unilaterally by the mesoderm which forces the ectoderm to expand correspondingly [2] (Fig. 92).

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[1] *Twitty* and *Elliott,* 1934.

[2] *Harrison,* 1929a.

Factors terminating growth abruptly.

The last size limiting factor to be considered is the *duration of growth.* Slower growth extending over a longer period can produce the same size as faster growth for a shorter time. The growth arresting factors are either intrinsic or extrinsic to the growing system. We have already discussed the intrinsic limitations of growth resulting from the gradual exhaustion of proliferating cells by differentiation and destruction. Frequently, however, growth is interrupted by extrinsic factors before it has become exhausted. This case is illustrated in the following example.

Example 91.[3]

The *metamorphosis of an amphibian* consists essentially of specific reactions of the larval tissues to an excess of thyroid secretion suddenly poured into the body. Normally this occurs when the larva has reached a certain state of maturity. Experimentally, it can be precipitated prematurely by feeding or injecting thyroxin or certain iodine compounds. Metamorphosis means the end of larval growth because the autolysis and resorption of old tissues outweighs the production of new ones. *Precociously metamorphosed specimens, therefore, turn out as dwarfs.* Although growth is renewed after the transformation, the sudden, if only temporary, interruption produced by the thyroid crisis may serve as a general illustration of the type of outside interference that may bring growth to an end.

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[1] *Twitty*, 1932.
[2] *Harrison*, 1931.
[3] *Gudernatsch*, 1912; *Romeis*, 1915; review: *Uhlenhuth*, 1921.

As another example, we cite the longitudinal growth of the skeleton in mammalian limbs. Ossification of the long bones starts from discrete centers, one in the middle and one at either end. The intermediate zones remain cartilaginous for some time. So long as these cartilaginous links between the bony portions are active, growth continues. Then, at some phase of life, probably again through the interference of some hormone, these plastic links are ossified, freezing, as it were, the bone into a rigid structure which admits of no further growth in length.

One could enumerate a still greater variety of methods through which growth rates are controlled and growth limits fixed, but the foregoing review of the principal factors seems to be sufficient to prove the thesis that *the agents determining growth and size are not one, but many*, and very diverse ones, too.

In view of this situation there is no sense to such questions as: "When is the size of an organ determined?" or, "what is the nature of the size determining factors?" These factors are too heterogeneous to be reducible to a single principle; they have little in common except their ultimate effect upon the mass of living tissue produced.

REINTEGRATION

The need for reintegration.

We have now followed the elaboration of the shape, organization, structure and size of the organism from its earliest beginnings into a phase in which most of the final characters have been laid down in such detail that the essential work of development may well be considered as done. Each part has gradually acquired such an amount of definiteness of determination that its further fate depends merely upon whether or not circumstances will be favorable for its running the full course. The phase of conquest, as it were, is over, and all there remains to be done is to consolidate the gains. This latter phase, though perhaps fundamentally of less importance than early embryogenesis, is, nevertheless, of considerable interest because during it the organism reaches its final perfection. It is then that the elementary and crude groundwork of embryogenesis is elaborated into finer details, and the necessary functional adjustments of the intricate mechanism are effected. This phase reaches far beyond the limits of the embryonic period proper into adult life. Its outstanding features are the *reintegration of parts* by the two secondary coördinating systems, the *humoral* system and the *nervous* system, and functional adjustments, frequently referred to as *functional adaptation.* Finally, secondary developmental processes occurring in postembryonic times, summarily called *regeneration*, are to be considered in this connection.

The meaning and necessity of *reintegration* will become clear if one stops to think of the disintegrating effect which the early course of development has on the organism as a whole. One remembers that, as the parts acquire definite structural and functional properties, they also become independent from one another and escape from common control. The emancipation of local fields, evidenced by the increasing self-differentiating power of individual germ fragments, is the most striking expression of the gradual decentralization attending embryonic development. *As development proceeds, the original unity of the germ breaks down.* Parts which formerly depended upon each other for specific assignments become increasingly immune to mutual interaction, and the designation of an advanced germ as a *mosaic* expresses the situation very poignantly. The initially unitary organism has become parcelled up into smaller units bound together only by spatial vicinity and by their dependence upon common food supply, oxygen supply, protection from the environment, and the like. To be sure, certain interactions between neighboring organs continue, and *mechanically* the germ is still a coherent system. However, the *dynamic* ties which during the early stages had subordinated practically every part to specific actions of the whole system have become severed, one by one. Now, as the organism approaches the functional phase, this dynamic disintegration becomes of vital concern; for *unity of action* and *concerted coöperation* of all parts in the service of the whole organism are prerequisites if the individual is to survive its embryonic phase. The very existence of the organism would be threatened, therefore, unless joint control of the individual members of the colony of organs could be restored. Furthermore, new communications among them must be established; for, one must bear in mind that, owing to the growth of the organism, the various organs have become spatially separated by distances immensely greater than those prevailing in the germ. It requires a nervous and vascular system to provide the channels of efficient communication over such long range.

A comparison of existing forms of animals indicates that the degree to which an organism becomes *decentralized* during development is directly correlated with its degree of *specialization*, which, in turn, is roughly correlated with its position on the evolutionary scale. In *protozoans* and most of the lowly *coelenterates* primary unity seems to persist throughout life. Evidence of this can be seen in their practically unlimited capacity for reconstitution and reorganization after injury, which proves that the harmonious play of developmental forces has not subsided. But, from these forms to the highly specialized and differentiated bodies of *vertebrates*, on which we have focussed most of our attention, it is a broad jump. Sweeping generalizations from one to the other seem unwarranted, and the assumption that higher organisms, too, retain their unity throughout development is not in agreement with the facts. It is in these *higher forms*, primarily, that the establishment of special integrative mechanisms has become a vital issue. The *humoral* system and the *nervous* system answer the demand.

HUMORAL REINTEGRATION

Hormones.

The *humoral* system consists of the body fluids which either circulate in formed channels (blood vessels and lymphatics) or diffuse freely in the body cavities and tissue spaces. This medium bathes all parts of the organism. In addition to food and oxygen supply, as well as waste products of vital activity, it carries *specific chemical substances* produced by certain types of cells and so devised as to have specific effects upon *distant* cells with which they come in contact. Substances complying with this definition are called *hormones* (see also p. 94). They are, in a sense, chemical messengers through which separated parts maintain communication. Their effects are predominantly of the *physiological* type, which is natural since their actions mostly fall into the functional period of the organism. But they also control many *developmental phenomena,* particularly during later phases. They figure prominently among the circumstantials under which growth is realized; specifically, one recalls in this connection the effects of the pituitary gland (example 7), of the hormones of the sex glands (example 3), of the hormone of the thyroid (example 91).[1] In all these instances, some morphogenetic or histogenetic feature could be linked with the presence, and frequently also with the dose, of a specific hormone. Some of the effects are irreversible, as the metamorphotic effect in amphibian larvae; others belong strictly in the class of physiological fluctuations producing merely histological "modulation," rather than differentiation (see p. 93 and Figs. 14, 15).

Are hormones primary factors of development?

The splendid work done in the field of hormones in the last decades has made us increasingly conscious of the almost ubiquitous intervention of these controlling agents during the *postembryonic* phase of the organism. How far their activities date back in the *embryonic* history is still to be decided. One knows that sex hormones prepared from adults can affect the sexual development of embryos to which they have been administered,[1] but it has not been ascertained whether these experiments reproduce, or merely simulate, the normal mechanism of sex differentiation. At any rate, there is some indication of hormone activity in later embryonic stages; besides, since there is no sharp distinction between embryonic and postembryonic events (see Part One), the dating of the onset of hormonal activity with reference to birth or hatching does not seem particularly logical.

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[1] For a comprehensive treatment of hormone effects on growth and later morphogenetic processes, consult *Van Dyke,* 1936; *Allen,* 1932.

It is another matter, however, to decide whether hormones are *primary* or *secondary* factors in development. Can they *produce* localization and specific differentiations, or are they merely *accessory* factors in the realization of these events? All present evidence points toward the latter alternative. Much depends, of course, on what one cares to define as a hormone. If, as is sometimes done, practically every chemical substance having any sort of an effect inside of an organism is called a hormone, the presence of such "hormones" from the very beginning of development becomes a matter of course, in view of the fundamental role of chemical activity during development. But there is no reason why one should not then simply speak of "chemical substances." If, however, hormones are understood to be *specific* substances of *local* origin, *diffuse* circulation, and again *localized* or otherwise *differential* action, then agents answering this definition cannot possibly play an organizing role, for the following reason.

It is inconceivable that a chemical agent diffusing indiscriminately through a body whose parts are all alike should ever be able to produce local differences. Just think of the process of etching. Acid poured over a homogeneous metal plate corrodes it evenly. To produce patterns, one must cover parts of the surface, thus protecting them against the attack of the acid. Or one may sensitize parts of the design so that they will become corroded more easily than others. In either case, the pattern as such antedates the action of the acid which brings it out. Or consider the photographic process. The developer does not create the picture; it simply converts a latent picture into a visible one. The picture is created when light sensitizes different parts of the emulsion differentially to the developing action in the dark-room. Whether or not development actually follows, is immaterial; potentially, the pattern is there, once the plate has been exposed. There is more fundamental difference between two undeveloped plates, one unexposed and the other exposed, both looking alike, than there is between two exposed plates, one undeveloped and the other developed, which differ so conspicuously in appearance.

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[1] *Willier, Gallagher and Koch,* 1937.

Hormones cannot produce organization.

In other words, wherever local differences arise in the wake of diffusing chemicals, they must have *virtually* existed in the reacting system *beforehand, as local variations of sensitivity and affinity toward the diffusing agent.* Thus, what makes such a system an organized system, as against a mere chaotic mass, is the latent pre-localization of differential *reactivities*, rather than the varying degrees to which these have become translated into manifest *reactions*. Fundamentally, therefore, the problem of *organization* can expect no elucidation from the study of the "dark-room" phase of the process.

Applied to the organism, this means that hormones, being diffusing agents, could never produce manifest differences among parts unless the parts were already latently distinguished from one another, at least, so far as specific and selective sensitivity to the hormone is concerned. Hormones expose and modify existing differences, but they cannot create new ones. Thus, they cannot be *primary* factors of organization. But, once the fundamental organization has been laid down, and parts have assumed distinctive properties, including hormone sensitivity, then, indeed, hormones rise to great prominence as instruments of *secondary reintegration* in that they bring the individual parts back under a common control. They activate and synchronize events widely separated in space; they "inform" the sensitive tissues of the state of their community, accelerate or depress their activities as needed, and, in general, serve to maintain functional equilibrium within the organism. A whole new field of research, *Endocrinology*, is devoting its efforts to the study of these phenomena.

NERVOUS REINTEGRATION

The role of the nervous system in development.

The other major instrument of reintegration of the body is the *nervous system.* Although primarily of physiological concern, it also exerts influences upon those later developmental phenomena which continue into the predominantly functional period of the organism. It must again be emphasized that this is a *secondary* control, established after all the essential features of development have been laid down by other, non-nervous, factors. Contrary to some beliefs which only a few decades ago were still in good standing, we know now, positively, that *all fundamental phenomena of morphogenesis and histogenesis are independent of those physiological actions of the nervous system which we call conducted impulses, or excitations.* As will be described more fully in Part Four, the non-nervous organs and the nervous system develop concurrently and only later enter into more intimate connection. Up to the time of the establishment of nerve connections, the nervous system has no control over the rest of the body, and, whenever it does affect the development of other parts, as in the case of nose, lens, and ear induction (p. 354), it does so by virtue of its organizing power as an embryonic field district, rather than by truly nervous excitation.

In earlier days of embryological science, the nervous system was sometimes rated as a more potent factor in development than it eventually turned out to be. Its integrative and coordinating function in the adult was known. It is only natural that one suspected it of playing a similar role in development. But this view could not long be maintained in view of an ever increasing number of opposing facts, some of which are outlined in the following.

Development is essentially independent of nerve influences.

The specific character of all organs is determined long before nerves make their appearance; thus, *segregation is unquestionably independent of nervous activity.* Histological differentiation of considerable perfection has been obtained in tissue culture and in interplantation (p. 360), that is, under conditions in which no nervous influence could reach the tissue. Thus, the major traits of *differentiation do not require the presence of nerves.* Even those tissues which later come under the most direct control of the nervous system, the *muscles* and *sense organs*, develop first in full independence. Explanted myoblasts differentiate into contractile muscle fibers[1] with cross-striation;[2] the capacity of self-

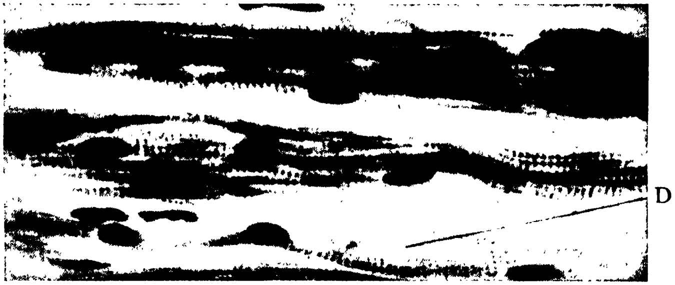

FIG. 96. Differentiation, followed by degeneration, of cross-striated muscle in the absence of nerve supply. (From *Hamburger*, 1928) Limb muscles experimentally prevented from receiving nerve supply have developed typically. (See also Fig. 74) Later regressive changes wipe out some of the earlier differentiations.

D, Degenerating area with fading cross-striation. × 460.

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[1] *Olivo*, 1925, 1928; *Holtfreter*, 1929, 1933d.

[2] *Goss*, 1933.

differentiated heart muscle (p. 253) to pulsate in the absence of all innervation has been the most convincing test of the purely muscular origin of the heart beat. Limbs, which have been prevented from receiving nerve supply by the experimental obstruction of nerve outgrowth, nevertheless, develop normal musculature (Fig. 96; see also Fig. 74).[1] Consequently, *the stimulus of functional activity* which is ordinarily mediated to the muscles by their nerves, *is irrelevant for muscular differentiation. Sensory organs* can likewise develop in the absence of innervation.[2] So can *bones* and *joints:* in explanted limb buds, joints form in their typical positions, notwithstanding the absence of all movement (Fig. 48).[3]

Trophic nerve influence on developed tissues.

The *ability of peripheral tissues to develop to full functional capacity in the complete absence of innervation* is, thus, an incontrovertible fact. This does not imply, however, that the tissues, once developed, can persist in this condition indefinitely. In fact, they rarely can, and in many cases the influence of the nervous system cannot be permanently withheld from them without serious effects. This is particularly true of muscles and sense organs. When a motor nerve is severed in an adult, the denervated muscle undergoes atrophy and degeneration. Its histological structure shows regressive changes, and its contractility is impaired. The same kind of changes, however, occur in muscles which have from the very start developed without nerves. As we have said before, muscles can develop normally regardless of a lack of innervation; but, if they remain nerve-free beyond a certain critical stage, definite signs of regression appear,[4] comparable to those following secondary destruction of the nerve supply in adults. In other words, *although the muscle can attain functional perfection without nerves, it cannot maintain it without their aid.* Apparently, some factor essential for the integrity of the muscular condition, and originally contained in the tissue itself, becomes exhausted or otherwise subsides, and must be replaced by an equivalent influence from the nerves. The same is true of *sense organs.* They can develop equally well with or without nerve connections (see above), but they *cannot retain their fully differentiated condition permanently, unless they become innervated.* This is indicated by the fact that many sense organs degenerate upon severance of their nerves.[1]

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[1] *Hamburger*, 1928.

[2] *Stone*, 1933.

[3] *Fell and Canti*, 1934; after the joints have formed, the bones fuse again, owing to the lack of movement.

[4] *Olivo*, 1928; *Hamburger*, 1928.

This *preserving* influence of nerves cannot be a product of their ordinary impulse-conducting activity, because, in the case of a sense organ, the impulse is conducted centripetally, away from the organ, while the preserving effect is exerted centrifugally, in the direction from the nerve cell body to the peripheral tissue. Nervous effects of this kind, which are essential for the maintenance of peripheral tissues but are not of the order of functional excitations, have been designated as "*trophic.*"[2] The possibility that the nerve releases specific substances into the tissue has been suggested.[3] Effects on tissue metabolism should also be taken into consideration. During later phases of development, the nervous system establishes trophic control over most tissues of the body including skin and bone. Nervous influences are also prerequisite for the *regeneration* of many organs (e.g., amphibian limbs) whose ontogenetic formation has been entirely free from such influence.

Nerves act on the tissues either directly or by regulating glandular activity, blood flow, etc. In the last analysis, even the muscular contractions and movements of joints mediated by the nervous system produce structural effects, so that true functional activity, at last, joins the array of developmental accessories, albeit in a relatively inferior role. This brings up the problem of "*functional adaptation.*"

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[1] *Olmsted,* 1920; *Brockelbanck,* 1925; *May,* 1925; *G. H. Parker,* 1932; see, however, *Stone,* 1933.

[2] Review: *Fleischhacker,* 1927.

[3] *G. H. Parker,* 1932.

FUNCTIONAL ADAPTATION

Adaptedness vs. adaptation.

The term "functional adaptation" is ambiguous, because it may imply the state of being adapted, as well as the act of becoming adapted. It may connote, first, the simple fact that the structure of every organ is well adapted to the functional requirements, or, second, that functional activity itself has been instrumental in fitting the structure to its use. The first point is a biological commonplace. This is by no means true of the second point. We are not considering here the appearance of adaptations in the course of *phylogeny;* it is a matter of record that of the two opposing concepts of evolution, the theories of natural selection and of direct adaptation, the latter has been steadily losing ground and has been reduced by the recent advances of genetics and evolutionary theory to almost purely historical interest. In the present connection we are concerned only with the question as to whether and to what extent there is direct adaptation of morphological structures to their functional uses during the history of the *individual.*

Functional hypertrophy.

Examples of some degree of *direct adaptation* can be found in every ontogeny, and it is from these examples that the old Lamarckian theory of adaptive evolution took its cue. *Hypertrophy* as a result of intensive functional use is probably the best known example of functional adaptation. The enhancing effect of exercise on the size of the muscles is familiar. It does not matter whether this is a direct effect of increased activity or an indirect result produced by the augmented blood supply to the active organs: in either case, it is the *function* itself which rebounds upon the organ structure, resulting in increased efficiency. Another example is the hypertrophy of one kidney as a result of the increased load of excretion in cases in which the other kidney has been removed.

It will be noted that, in these instances, function only affects the size of existing organs without creating novel structures. The essential development of the *muscle* is entirely *prefunctional,* and all that later function can do is to regulate its size within certain limits. The development of the *kidney* is, likewise, *prefunctional* in all its major aspects, and its function retains only some degree of control over the final size. Similarly, it has been found that the length of the *intestine* in amphibians changes according to the kind of diet; the less digestible the diet, the more the gut grows in length.[1] Functional use, therefore, must be added to the list of circumstantials upon which growth rates and growth limits depend.

Structural adaptation to mechanical function.

There is another group of phenomena, however, in which the morphogenetic effect of function is much more subtle and, indeed, *pattern determining;* they are observed in the supporting tissues of the organism — *connective tissues, cartilage,* and *bone* — whose texture and arrangement are so perfectly adapted to their mechanical function that even the most meticulous construction engineer could find no fault with them. Let us consider, for instance, the *tendon,* which serves to transmit pull from the muscles to the skeleton.

A *tendon* consists of numerous tough, fibrous strands running strictly parallel in a lengthwise direction (Fig. 97A). Their orientation in the direction of pull insures the greatest mechanical efficiency because the tensile strength of a fiber is greatest in the direction of its axis. This fact, as such, merely proves that the structure of a tendon is well suited for the mechanical function for which it is used. However, this same adaptive structure is resumed in tendon *regenerates,* and there one can prove that it is molded by actual mechanical function.

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[1] *Babák,* 1903; *Klatt,* 1926.

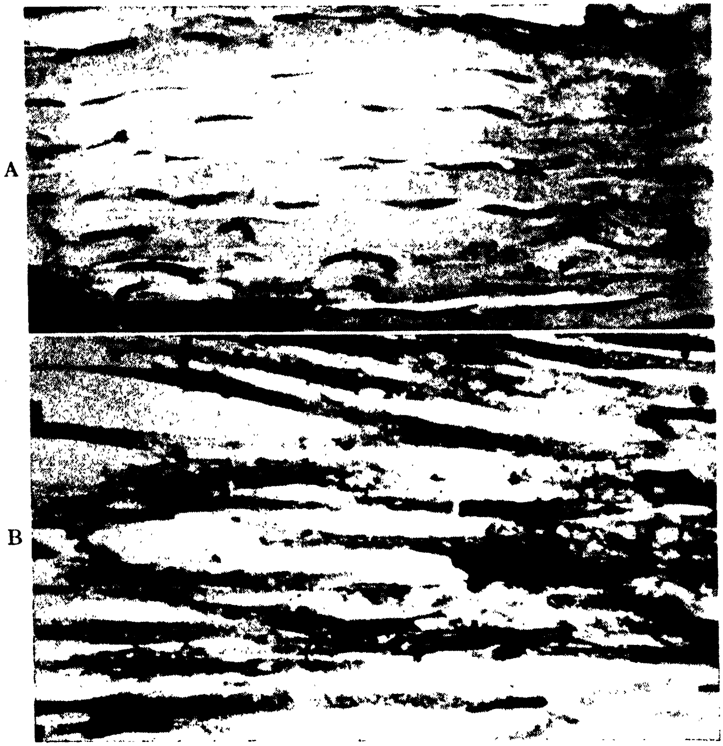

Fig. 97. Tendon structure as mechanical effect.
A, Longitudinal section of rat tendon. Note parallel orientation of fibers and cells.
B, Embryonic mesenchyme (chick) cultivated in vitro in plasma membrane under tension (see Fig. 98). Cells assume shape, proportions and orientation similar to tendon cells. × 495.

Example 92.[1]

When a *tendon* is severed, a scar forms between the gaping ends. This scar tissue is, at first, very irregular, but, under the influence of the tension continually exerted upon it by the contracting muscle, its cells and fibers soon assume a *lengthwise orientation in the direction of the pull,* thereby gradually restoring the typical texture of the tendon. An experimental change in the direction of the pull causes a corresponding disorientation of the resulting tendinous structure.[1]

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[1] E.g., *Lange,* 1917; *Nageotte,* 1922.

Functional adaptation in vitro.

Thus, mechanical pull has produced a definitely adapted structure. This case is of particular interest because it is one of the few instances in which the mechanism of pattern formation could be duplicated and analyzed in tissue grown in vitro, removed from the influences of an organism. As was described above (p. 423), mesenchyme cells explanted into a clot of blood plasma grow out into the medium in more or less random fashion (Fig. 22), and the cells assume no definite orientation. Such cultures, therefore, supply excellent test material for the identification of supposed orienting factors, such as *tension*, which from observations on tendon regeneration and similar experiences was suspected of being such an orienting factor.

Example 93.[2]

Mesenchyme cells from a chick embryo were explanted into a specially prepared medium: the blood plasma, instead of being placed on a slide, was stretched in the form of a very fine membrane over a small glass frame. This set-up produces tensions in the medium which are oriented along definite and predictable lines. In a triangular frame, for instance, the main tensions are perpendicular to the three sides. *Cells growing out under these conditions followed distinctly the lines of stress* (Fig. 98). The elongate cells were rigorously oriented and drawn out in the direction of these lines. This strict orientation, furthermore, enhanced their migration and growth. In fact, the entire texture of the newly formed tissue varied with the local pattern of stress. Where the tension reached a maximum, the tissue then assumed a configuration which resembled strikingly that of a tendon (compare A and B in Fig. 97).

Thus, the *directive effect of oriented tensions* on tissue growth and texture has been reproduced in vitro. Continued experiments have also revealed the manner in which tension orients cells. Primarily, it acts on the medium. Blood plasma contains polar, rod-shaped ultramicrones of fibrin which during coagulation aggregate into a spongy framework. When the coagulum is subjected to tension, the mechanical force, according to the statements made on page 34, gradually orients the ultramicrones into a parallel alignment so that the aggregates all become oriented in a common direction coinciding with the lines of stress. These oriented aggregates serve, secondarily, as a network of guiding rails to the cells; the latter follow the oriented tracks and thereby acquire a corresponding orientation (Fig. 99). *Tension, therefore, does not affect the cells directly, but acts by producing an oriented structure in the colloidal medium which the cells then retrace.* Because the colloidal structure remains, at first, ultramicroscopic, it has been designated as an "*ultrastructure.*" There is evidence that the events as analyzed

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[1] *Levy*, 1904.

[2] *Weiss*, 1929.

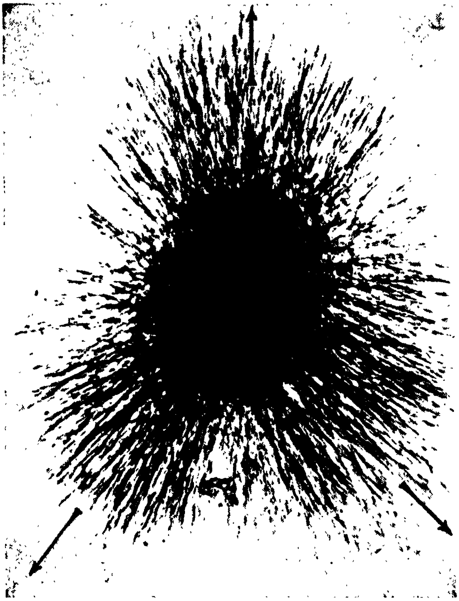

FIG. 98. Effect of tension on cell growth in vitro. (From *Weiss*, 1929) Embryonic connective tissue cells cultivated for 3 days in a film of blood plasma subjected to tensional stresses in three directions indicated by arrows. Note the strict orientation of cell growth and the increase of cell density in the directions of maximum tension. Compare with the uniform growth of Figure 22.

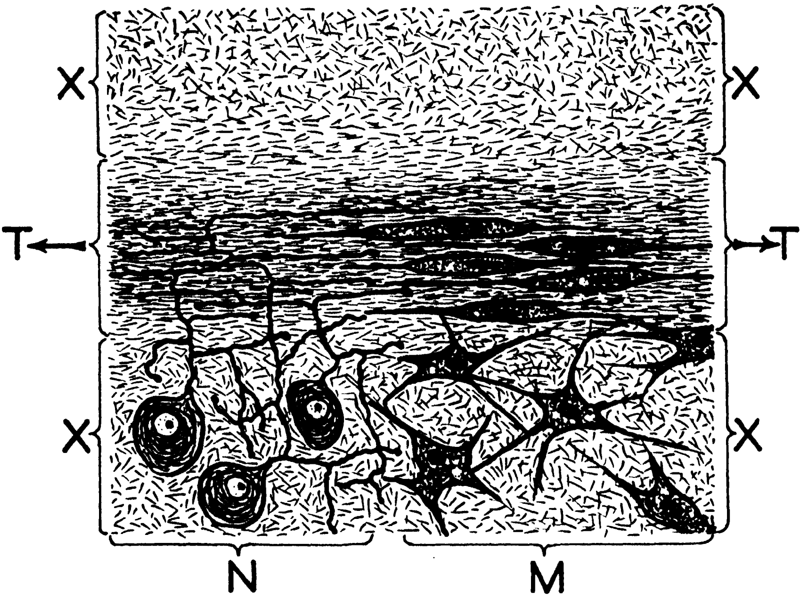

FIG. 99. Effect of the ultrastructural organization of the medium on the orientation and shape of cells. The picture represents a colloidal medium with rod-shaped ultramicrones (see p. 33). The orientation of the ultramicrones is symbolized by dashes (out of scale!). The middle part of the medium has been stretched in the direction of the arrows (T–T), whereby an oriented ultrastructure has been produced (see p. 34). Unstretched medium (X) lies to either side of this oriented strip. Nerve cells (N) and mesenchyme cells (M) are present in the lower portion. Those cells and nerve fibers which have invaded the oriented median part have assumed corresponding orientations (cf. Figs. 97 and 118).

in tissue culture faithfully reproduce the analogous situation in the organism.[1]

Ultrastructural organization as basis of tissue structure.

It can readily be seen that these results, in addition to elucidating the mechanism of functional adaptation in connective tissue, bear directly on the wider problem of cell orientation and tissue pattern in general. They stress the *directive role of the colloidal matrix* in which the cells live. They demonstrate how profoundly the pattern of this matrix affects the orientation, movements and grouping of the cells.

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[1] For a review of the problem, see *P. Weiss*, 1933.

Accordingly, every factor contributing to the organization of the matrix must be viewed as having, eventually, some influence upon the organization of the tissue arising in that matrix. In this light, the fact that it happened to be just tension which produced the effect in the above examples is immaterial. For many other physical agents can be enumerated which are no less effective than tension in forcing aggregates of colloidal ultramicrones into definite orientations (see p. 34). Orientation and aggregation, in turn, affect the electric state, direction of capillary currents, retention and release of intermicellar fluid, adsorption, chemical surface reactions, and other properties of the medium, many of which will further modify the state of the cells exposed to them.

There can be no doubt, therefore, that ultramicronic (micellar) orientation (*"ultrastructural organization"*) is one of the fundamental instruments of tissue organization; in fact, its operation is demonstrable as early as in the unsegmented egg where presumably oriented arrangements of the colloidal ground-substance of the cytoplasm point the way to the first orderly displacement and segregation of substances of distinctive properties. From segmentation on, the intercellular matrix, whose character and developmental history have been outlined on pages 56 and 73, serves in the function of guiding ground-substance. This matrix obviously becomes the intermediary between the different kinds of directive agents on the one hand and the execution of directed movements and other consecutive transformations of the individual cells on the other. It converts forces into structures. The colloidal structures are at first plastic and modifiable and susceptible of regulative reorientation (see p. 33); after reaching a critical limit of consolidation, however, they become rigid and unadaptable. This course parallels the general ontogenetic trend of the tissues from "regulative" behavior to "self-differentiation." Thus the view gains support that much of the *texture of the organism* *can be ascribed to orienting forces which create ultramicroscopic patterns in the colloidal matrices.*[1]

The functional fitness of bone structure.

Returning to the more specialized subject of "functional adaptation," perhaps the most spectacular example of the direct effect of function on tissue architecture is the *bone.*

Example 94.

A longitudinal section through an extremity reveals that the long bones are compact only in their outer layers (so-called *substantia compacta*), while further inward they consist of a delicate framework of ossified trabecles, the so-called spongy bone (*substantia spongiosa*). It has long been recognized that the interlacing lamellae of the latter are arranged, not at random, but very systematically; they form a pattern of definite lines which, according to mechanical engineers, *correspond to lines of mechanical stress.*[2] If a technical designer were confronted with the task of building a trestle of the shape and the load of the bone in such a manner as to achieve maximum constructive firmness, he would place braces in precisely the orientations in which the bony trabecles are aligned. The thigh bone, for instance, is built according to the principle of a crane (Fig. 100). The weight of the body rests upon the protruding femur head and produces complicated stresses of tension and pressure inside the bone. Any crane must be fortified against collapse by bars arranged along the lines of maximum tension which can be calculated for a given shape and direction of load; these braces, then, take up the stresses arising from the load. A construction of these lines on purely technical principles for a crane of the shape of the bone leads to a system of lines (so-called trajectories) of a pattern essentially similar to the one actually observed in the spongy bone of the femur (cf. Fig. 100 A and B).

The fact that the architecture of the tissue meets precisely the demands placed upon it by the functional stresses

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[1] The importance of the organization of the "ground-substance" for the texture of tissues has been illustrated and emphasized by *Nageotte* (1922); see also *Baitsell,* 1921, 1925.

[2] For a thorough treatment of the subject, see *Murray,* 1936.

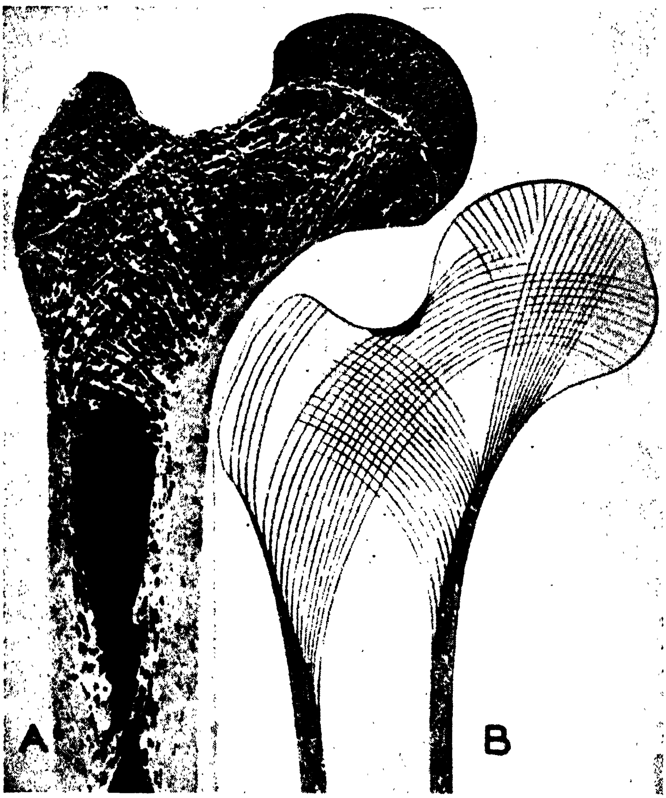

Fig. 100. Trajectorial structure of bone. (After *Wolff*)
A, Longitudinal section through the head and shaft of a human femur showing the pattern of trabecles in the spongy bone.
B, Reproduction of the main lines of the trajectorial pattern.

is, at first, merely an expression of fitness, rather than of actual fitting. But again, as in the case of the tendon, we possess evidence that function itself can mold such patterns directly, at least, during later stages of life.

Example 95.[1]

Transformation of bone in response to mechanical change.

Certain diseases entail ossification of joints. A knee joint thus afflicted is replaced by a bony bridge which fuses the leg bones firmly to the thigh bone. If the fusion occurs under an angle (Fig. 101), the whole skeleton of the limb consists of a single arched bone continuous from hip to ankle. The stresses to which this bone is subjected every time the body weighs down on it can be easily visualized by bending a properly shaped strip of rubber: the convex side becomes stretched and the concave side compressed; tensions near the former are oriented tangentially, near the latter, radially. Figure 101b indicates the distribution of trajectories in a bone thus loaded, and Figure 101a shows the actual architecture of the spongy bone which has been observed in such patients. The *correspondence between tissue orientation and lines of stress* is obvious.

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[1] *Roux*, 1885; *Wolff*, 1892.

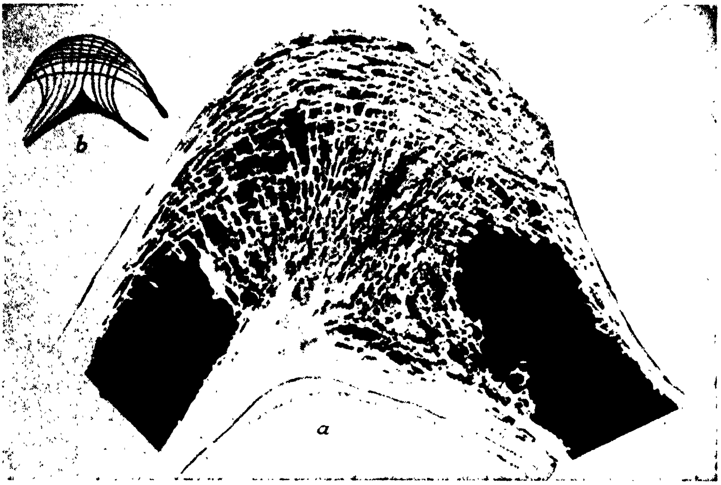

FIG. 101. Transformation of bone structure.
a, Longitudinal section through an ossified human knee joint (ankylosis), showing the structure of the spongy bone which has formed between the formerly jointed femur and tibia. (From *Roux*)
b, Diagram of main trajectories.

Where we now find a continuous system of trajectorial lamellae, there used to be a knee joint: two compact ends of bone separated by a gap. One realizes, therefore, that what actually has taken place, is not only the formation of new bone but, a *complete reorganization* of the interior of the old bone as well; and this whole reorganization bears the unmistakable impress of the pattern of *mechanical stresses* to which the bone was subjected in the abnormal situation. The old bone has converted its structure into one better adapted to the new mechanical situation which has arisen from the fusion.

The origin of the embryonic structure of bone.

This is, indeed, functional adaptation, not merely functional adaptedness. Immediately, the question arises as to whether the *normal* architecture of the spongy bone is likewise the result of mechanical tension and pressure effects. A study of embryonic bone shows that the essential spongiosa pattern is already formed long before there is any bearing down of the body on the bone. This fact eliminates, of course, the possibility that the internal structure of the bone might be a result of its supporting function. But this does not imply that tensions of other than functional origin might not be involved. In fact, there are indications that the pattern of the trabecular framework actually is a result of tensions, namely, those internal stresses which arise in the developing skeleton owing to its *differential growth*, and, hence, unequal expansion.[1] Since the bone is always deposited upon a foundation of connective tissue or cartilage, the primary effect of the growth tensions evidently consists of the imposition of trajectorial structure upon these tissues; this structure, then, secondarily, serves as a model for the forming bone.

Why the growth of the skeleton occurs in such a way as to provide a serviceable structure for its later function, is a question which cannot be answered by the study of embryology. For a solution, one must turn to principles transcending the history of the individual, e.g., evolution (see pp. 366, 558).

Summary: The formative and conservative phases of development.

The preceding discussion of "functional adaptation" can be summarized as follows. Development is essentially *pre-*

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[1] *Triepel*, 1922; *Rhumbler*, 1914; see *Murray*.

functional and *pre-adapted;* that is, organs and tissues develop their specific structural equipment and functional capacity entirely without the aid and intervention of that functional activity for which they are specifically built. However, after embryonic processes have once established structures capable of functioning, functional activity itself may gain some control both over the maintenance of these structures and their reconstruction after disturbances (examples 92, 95). This, in conjunction with the fact illustrated above that nerve and hormone actions also take secondary charge of the preservation of erstwhile non-functional differentiations (e.g., muscle), suggests that the development of most organs and tissues passes through two phases, one of *formation* and one of *conservation,*[1] each under the prevailing control of a different set of factors.

REGENERATION[2]

Regeneration is true development.

In our discussion of embryonic development, we had repeatedly occasion to comment on the fact that the developmental factors producing a given structure do not generally become exhausted in this act (see p. 318). Their persistence and capacity for repeated action form the basis of the phenomena of *reconstitution* and *regeneration.* Regeneration is the repair by growth and differentiation of damage suffered by an organism past the phase of primordial development. A brief consideration of *regenerative processes* will disclose that they are *fundamentally of the same nature and follow the same principles as the ontogenetic processes.* When an excised piece of tissue or an amputated part of an organ is regenerated, the procedure does not essentially differ from that followed during first development, notwithstanding minor deviations. The capacity for regeneration is, therefore, not a novel and secondarily acquired adeptness of the organism at meeting later accidents by adequate repairs, but is simply *a residue of the original capacities for growth, organization, and differentiation* through which the individual was first formed. Hence, the extent of regenerative capacity is limited by the extent to which formative capacities survive the ontogenetic phase.

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[1] Both *Roux* and *Braus* have emphasized the existence of these two phases.

[2] An exhaustive monograph on Regeneration has been published by *Korschelt,* (1927). To this the reader may be referred for further information.

Extent of regenerative capacity.

In some forms regenerative power is enormous, while in others it is almost nil. On the whole, one gains the impression that *regenerative capacity tends to vary inversely as the scale of organization.* Generally speaking, the percentage of good regenerators is lower among the higher forms than among the lower, more simply organized, forms. But, when it comes to particulars, there is not a single group of animals whose regenerative capacity could be safely predicted from its position on the evolutionary scale alone. There are lowly forms with practically no regenerative capacity, for example, the *ctenophores* and *rotifers;* while, on the other hand, some incomparably higher forms, such as the *crustaceans* and *amphibians,* regenerate amazingly well. Moreover, closely related forms, such as the earthworms and the leeches, or the tailed and tailless amphibians, may often represent diametrical extremes with regard to regenerative capacity: the earthworms and urodele amphibians are excellent regenerators, while the leeches and anurans are among the poorest.

Physiological repair.

Some regeneration is observed in all forms in which growth and the proliferation of new cells continues throughout life. In such forms the cells which are incessantly produced to compensate for the steady physiological loss of differentiated cells through wear and function (p. 424), on occasion, become available for more extensive replacements. Owing to this fact, even the adult human individual can repair a moderate loss of skin, connective tissue, muscle and bone — tissues in which proliferation has never come to a complete stop (see p. 13). The cells engaged in such repairs have usually no wider potency than that of reproducing their own type; they are no longer totipotent and cannot be made to enter a course of differentiation different from the one they had been pursuing previously. The only margin left is that of *modulation* (p. 93) which permits cells at a definite level of differentiation to assume a variety of forms and states depending on the environmental conditions under which they exist. Mesenchyme cells, for instance, can appear as free blood cells or as fixed connective tissue cells, and, under certain conditions, they can even produce bone. A transformation of this order is known in pathology as *metaplasia.*

Organ regeneration.

However, when the loss is still more extensive, especially if it affects highly complex organs, more comprehensive powers are necessary to repair the damage. *Regeneration of an organ* is, thus, on a different level from mere restitution of an injured tissue. The former requires not only the capacity to grow, but in addition the presence of factors which will lead the growing mass into the proper differentiation and organization. In the following we shall confine our attention to these more comprehensive processes of *organ regeneration.*

Numerous invertebrate forms are known in which *major portions of the body can be repeatedly replaced* after ablation (see p. 373). We find such forms among *hydroids, flatworms, nemerteans, annelids, bryozoa, echinoderms,* and *tunicates.* In most of these a fragment measuring only a small fraction of the original body can reconstitute a complete individual. The regenerative capacity of *vertebrates* is much less extensive. Even in the most favorable cases, it is confined to the *restoration of organs.* We choose for our discussion the group which has been best explored analytically: the *amphibians.*[1]

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[1] The following brief report does not even attempt to sample the field of regeneration phenomena adequately. Instead, one specific problem, namely,

Regenerative power is very high during the larval stages of all amphibians; and in urodeles, it persists even after metamorphosis. These animals can adequately replace parts removed from the following organs: eyes, jaws, limbs, gills, tail, and a number of internal organs. When one considers the great complexity of an organ structure, such as that of an eye or a limb, and further takes into account that what is reproduced through regeneration is not always a whole limb or a whole eye, but just the missing portion of these, one becomes aware of the intricacies of organ regeneration.

The prerequisites of organ regeneration.

In order that an organ may regenerate, the following conditions, among others, must be fulfilled. There must be a *stimulus* to provoke renewed formative activity. There must be *cellular material* with a sufficient store of potencies to produce all the histological differentiations necessary. There must again be *organizing factors* to make the right differentiations appear in the right places, to cause the proper alignments, movements, and functional transformations of the cells, to direct the growth of the reconstituted parts into the *proper proportions*, and to control the resumption of *functional activity* in joint coöperation with the old parts. Finally, there must be an adequate *supply of food* and other *vital necessities.* These prerequisites are essentially of the same order as those encountered in our study of ontogeny. Let us now consider them individually in order to determine whether they are actually the same.

The sources of regenerative material.

The first question concerns the *sources of regenerative material.* It was once surmised that regeneration of a complex organ is effected by the combined growth of the individual tissues of which the organ is composed; each tissue would reproduce its own kind and the various components would eventually be fitted together and assembled into a complete

the regeneration of the amphibian limb, has been selected to serve as a general illustration of the parallelism between regeneration and ontogeny.

organ. According to this theory, the regenerate arises from as many sources as the remainder of the organ contains tissues, and, if this were correct, regeneration would not involve such real diversification among cells as occurs during ontogeny. This strictly "preformationist" notion of regeneration has failed to receive support by experiments devised to put it to a crucial test. These experiments have shown that in organ regeneration, as in organ ontogeny, *the formative materials frequently are derived from a common source and undergo true differentiation de novo.*

A description of limb regeneration.

When the *limb* of a newt or salamander is amputated at the shoulder or at a more distal level, the cut traverses the following tissues: epidermis and cutis of the skin, connective tissue, muscles, tendons, skeleton, nerves, and blood vessels. After the operation, the free border of the epidermis moves over the wound and closes it. Within a few weeks, a mass of cells accumulates directly beneath this epithelial covering and forms a little cone, which is called the *blastema.* The blastema grows in size. By and by it assumes more definite shape, faintly indicating the outlines of the future limb. Eventually, the different segments of the limb and the fingers become distinct. Concomitantly, histological differentiation proceeds in the interior, and new skeleton, new muscle, and new skin differentiate. Nerves and blood vessels grow in from the base. In the end, after a period varying with the age and state of the individual and with the conditions of the environment, one finds again a fairly typical limb in the place of what had been an amputation stump.

Evidence against tissue specific origin of regenerates.

In order to test whether or not the individual tissues of the regenerate are each derived from the corresponding old tissue of the stump — bone from bone, muscle from muscle, and cutis from cutis — the following experiments were performed.

Example 96.[1]

When all the bones are cleanly extirpated from the developed limb of an adult newt, the operated limb remains permanently boneless and limp. The reason for this failure of the bone to be regenerated will be explained presently; for the moment, we will take it for granted. When such a limb is later amputated at the elbow level, neither the cut nor the rest of the stump contains bone. Nevertheless, a blastema forms as usual and gradually produces a *new distal part of a limb, equipped with its typical skeleton;* this, in spite of the fact that the old tissues of the stump have remained devoid of skeleton (Fig. 102).

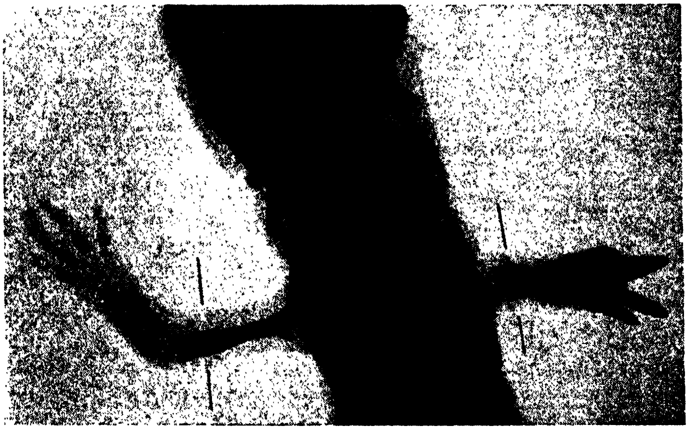

FIG. 102. Regeneration of limb from boneless base. (From *Weiss*, 1925) After extirpating the whole shoulder and arm skeleton on the right side both arms were amputated at the levels indicated by lines. The amputated parts regenerated and developed a normal skeleton from both the boneless and the bone-containing stump. X-ray photogram 11 months after operation.

It is evident that, inasmuch as there was no old skeleton present in the stump, the regenerated bone cannot have originated from old bone. It must have differentiated from the blastema. Analogous experiments in which other tissues were tested have led to essentially the same results.[2] All mesodermal parts of the regenerate seem to arise by differentiation in situ from the blastema, irrespective of whether or not the stump contains a corresponding tissue component. We must conclude, therefore, that the blastema is not an assortment of differentiated cells collected from independent contributions of the old tissues, but a mass of *equivalent* cells which later differentiate in different directions.

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[1] *P. Weiss*, 1925b; *Bischler*, 1926.

[2] Further literature in *P. Weiss*, 1930.

Regeneration blastemas of local origin.

Where do the cells of the blastema originate? There are two possibilities. They may be of local origin, cells mobilized from the vicinity of the cut; or they may be carried into the wound from a distance by the blood and lymph streams. Experiments have decided in favor of the former view.

Example 97.[1]

> Pseudo-parthenogenetic amphibian larvae, that is, larvae developed from eggs which had been fertilized with radium-killed sperm (p. 158), contain in their cells only the *haploid* chromosome number. The haploid cells can easily be distinguished from normal diploid cells by the considerably smaller size of their nuclei (p. 131). Taking advantage of this fact, limbs were grafted from haploid individuals to normal diploid hosts. After a graft had healed, its distal portion was again amputated so that only a small slice remained attached to the host body. From this slice a new limb regenerated. When this was later studied histologically, it was found to be *entirely composed of haploid cells*, although its blood supply had come from the diploid host.

The whole regenerate, therefore, has been built up by cells which originated *within the remnant of the grafted limb*, without receiving any tissue contribution from the host.

When the *lens* is extirpated from the eye of a urodele amphibian, a new lens is regenerated; the new cells are furnished either by the border of the iris (Fig. 103),[2] or by

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[1] *G. Hertwig*, 1927.

[2] The mode of lens regeneration in amphibians, first described by *Colucci* (1891) and *G. Wolff* (1895), has attracted considerable interest; see *Sato*, 1930, 1933.

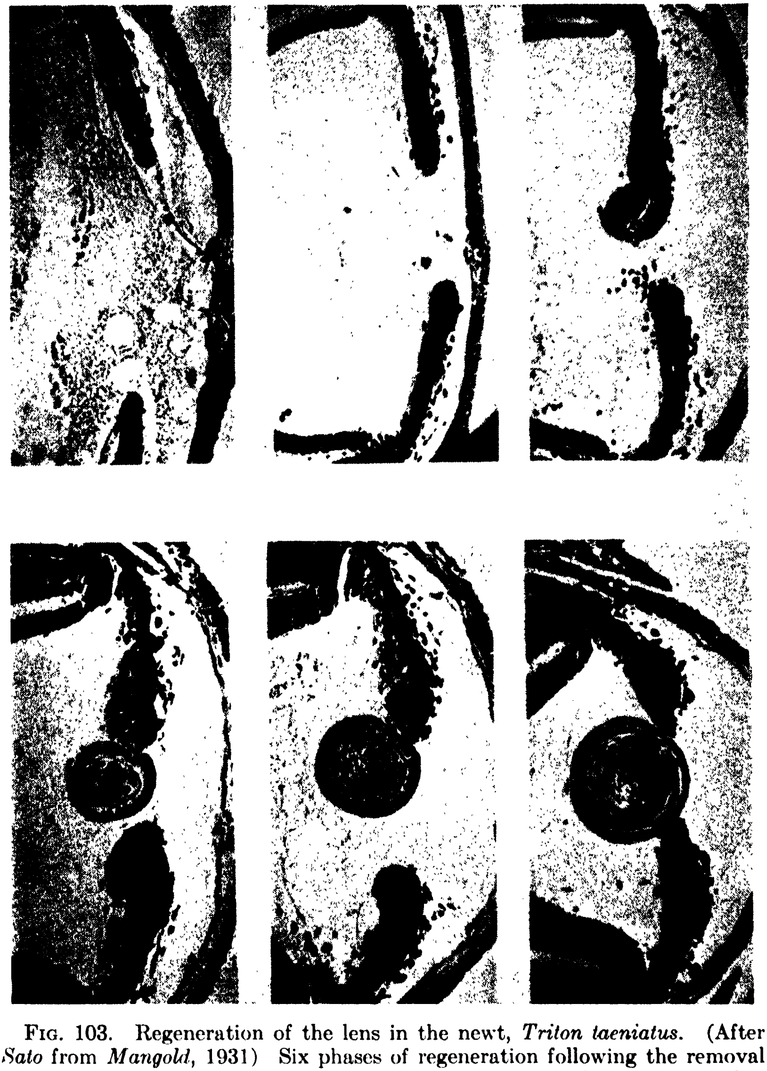

Fig. 103. Regeneration of the lens in the newt, *Triton taeniatus*. (After *Sato* from *Mangold*, 1931) Six phases of regeneration following the removal of the lens from the developed eye. The upper margin of the iris furnishes the new lens. The pictures represent stages 7, 10, 14, 17, 19, and 21 days after the extirpation. × 78.

the retina. Again the regenerative material is of *local* rather than of *distant* origin.

An extension of these results to other forms seems, however, inadvisable. In *annelids*, for instance, it has been observed that certain indifferent cells (so-called *neoblasts*) migrate over great distances to an amputation surface to serve there as blastemal elements.[1] Apparently, there is little uniformity in the methods of gathering cells for the blastema. Only the fact that a blastema is formed at all, is common to all kinds of organ regeneration.

The problem of cell dedifferentiation.

Let us, however, stay with the amphibians. Here the blastema cells clearly arise in the proximity of the wound. Their exact source is all but clear, since there is a variety of tissues in this vicinity. Two possibilities come to one's mind. Either differentiated cells of various types of tissues *dedifferentiate,* that is, give up their specialized characters and return to a more primitive condition, regaining at the same time wider potencies; or the blastema is produced by *reserve cells,* that is, cells whose previous differentiation has been arrested at some inferior level, and which, in this quiescent condition, have lain interspersed with the specific, more highly differentiated cells of the tissues. The question is of considerable importance, as it touches on the general problem of *reversibility of cell differentiation.* The problem is not so much whether cells can and do dedifferentiate as whether they can afterwards redifferentiate in a new direction.

We know, of course, that once they have been firmly set on a definite course of differentiation, cells persist in it even against the discordant tendencies of a strange location (p. 312). This fact as such, however, does not reveal whether the unrealized potencies of the cell are being *lost* or merely *obscured* during differentiation. Unquestionably, the specialized cell has become single-tracked. But has it become so because of a loss of its former wide potentialities, or perhaps only because all cell substance available for differentiation was forced into one particular kind of service? Maybe a developed muscle cell could still transform into a nerve cell, if only the contractile fibrillae and other accessories could be cleared away to give the protoplasm a chance to form a nervous apparatus instead. However, the facts indicate that the decline in potency attending the differentiation of a cell is real, not merely apparent. *Cells which have differentiated into different types have become intrinsically, not only outwardly, different.* The most conclusive evidence has come from tissue culture experiments.

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[1] E.g., *Krecker,* 1923; *Hämmerling,* 1924; *Stolte,* 1936.

The case against true dedifferentiation.

From the very beginnings of tissue culture it has been noted that many explanted cells gradually lose their former differentiated aspect and distinguishing cytological features.

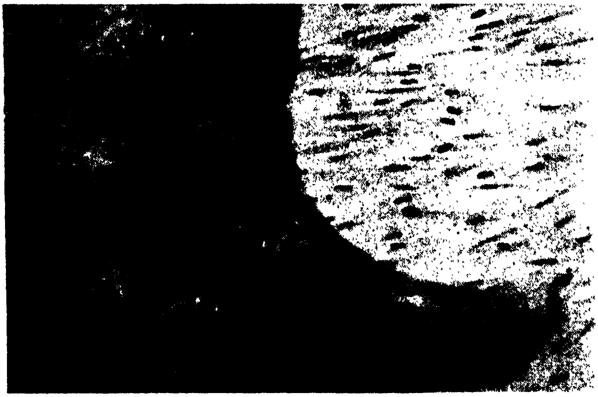

FIG. 104. Mixed tissue culture of epithelium (epidermis) and mesenchyme (connective tissue) of a chick embryo. (Original) The epithelial cells (with round nuclei) grow as a flat pavement membrane, while the mesenchyme cells (with elongate nuclei) grow in loose strands. × 180.

Eventually, all cultivated cells appear in only three forms: closely packed epithelial cells, loosely connected mesenchyme (Fig. 104) and free amoeboid cells. Originally, it had been thought that this abandonment by the cells of their specialized aspects meant a real reversion to a more primitive level of differentiation, and that identical appearances were indicative of identical character. Continued studies, however, have made it clear that the character of a cell cannot be judged by external appearances. Although superficially they resemble one another, the *explanted cells obstinately preserve many of their functional distinctions* even over prolonged periods of cultivation in vitro. Paraphrasing in human terms, one might say that cells, transferred into the simplified conditions of tissue culture, divest themselves of the manifold elaborate garments and tools which they used to wear in the organism, but retain their personal identity.[1] Cultivated glandular cells of the intestine and pigment cells of the eye may become very similar in shape and general conduct, but under proper conditions the former still resume the production of digestive enzymes, and the latter that of black pigment. The morphological simplification of cells in vitro is merely another instance of modulation.

Thus, tissue culture, which at first had been most suggestive of the occurrence of actual dedifferentiation of cells, has in the end furnished the strongest evidence against it. It seems safe to state, therefore, that *differentiation of cellular character in a vertebrate is irreversible;* this means that after it has once assumed a definite course of differentiation, a cell is never again able to switch into a different course. There is dedifferentiation of structure, but not of character. Only modulations are reversible.

Regeneration cells as reserve cells.

In view of this fact, it is very unlikely that the cells forming a regeneration blastema should to any great extent be cells of former high specialization. Nevertheless, the existing evidence is not adequate for a final decision. Histological observations indicate that a large proportion of the blastema is made up by *indifferent* elements which have been mobilized from the vast reservoir of unspecialized cells contained in the various connective tissues of the stump.[1] Cells from destroyed muscle [2] and skeleton have also been traced into the blastema. But, since it is impossible to say whether or not they ever contribute to any structures other than muscle and skeleton, respectively, their participation in the blastema neither proves nor disproves the irreversibility of differentiations. It is not unlikely that the tissues of the regenerate newly differentiating *in situ* are supplemented by differentiated cells immigrating from the stump and each joining their own kind.[3]

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[1] *Fischer*, 1929; *Törö*, 1934.

Whether cells of lower forms can dedifferentiate and then enter new lines of differentiation, is a question which must be settled for each group of animals separately, because the degree of specialization reached by the cells varies among different forms. One favorite case, however, which used to be cited as evidence for the permanent equipotentiality of differentiated cells in invertebrates, has lost its convincingness under more careful scrutiny, as follows.

Example 98.

The regenerative capacity of some tunicates is so high that a small fragment of the branchial chamber can reconstitute a complete and proportionate animal.[4] The older contention was that in this process the specialized tissues of the body wall first dedifferentiate into a homogeneous cell mass and then redifferentiate harmoniously into the various tissues of the new body. More recently, however, it was found [5] that there is no such cycle. *Reconstitution is effected solely by undifferentiated reserve cells* which can be easily spotted in the intact individual by their elective reaction to vital stains. When an animal is mutilated, these reserve cells furnish the entire material for regeneration of a new individual, while the old differentiated cells contained in the fragment disintegrate.

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[1] *Fritsch*, 1911; *Hellmich*, 1931.

[2] *Thornton*, 1938.

[3] *Goetsch* and *Hellmich*, 1931.

[4] *Driesch*, 1902.

[5] *Spek*, 1927.

In other words, the cells building the regenerate are not transmuted tissue cells, but spring from a stock of undifferentiated elements. The situation is, therefore, much the same as in amphibians. It is possible that other forms behave differently, but in view of the importance of the question more definite proof than has thus far been forthcoming must be awaited before general conclusions can be drawn.

A comparison between regeneration blastema and organ rudiment.

Returning now to the subject of regeneration, the fact that the blastema is simply an unorganized herd of cells, raises the question of how this mass acquires the definite organization necessary to build a typical organ. On the whole, a *regeneration blastema* can be compared to an *embryonic organ rudiment.* In both cases, the equipotential character of the cells can be demonstrated by the same methods, namely, experimental alteration of the material. Up to a certain critical time after the beginning of regeneration, arbitrary amounts of the blastema can be removed without causing defects to the limb developing from the remainder.[1] Conversely, when part of one early blastema is added to another, the combined mass builds up a single harmonious limb.[2] All of this goes to show the *versatility of the cells of the young blastema;* they differentiate in accordance with their varying positions in the artificially reduced or enlarged systems.

But just as in the ontogeny of an organ rudiment the state of indeterminacy of the cells is gradually superseded by a state of definite determination in which every part pursues a definite and irrevocable course, so the blastema likewise lapses into a "determined" condition as it grows older. *At this later stage, local damage inflicted upon the regenerate entails a corresponding local defect in the end product.*[3] Thus, the time at which different portions of the blastema have acquired self-differentiating capacity has been ascertained.

[1] *Schaxel,* 1922; *Swett,* 1928c.

[2] *Weiss,* 1925c.

[3] *Schaxel,* 1921.

Studies on regeneration concur with those on embryogenesis also in demonstrating that what we call "determination" is neither a single nor a sudden event. It progresses gradually from the general to the specific. At a time when the blastema already possesses self-differentiating capacity as a whole, its individual cells are still permutable. It is conceivable that in a limb regenerate a few primordial foci of qualitative differentiation are laid down first, for instance, one for each skeletal unit and one for each muscle, and that these advanced centers then continue development by recruiting cells from the pool of undifferentiated reserves available in the blastema. These processes have really not yet been sufficiently explored to permit a definite opinion. But one thing has become evident, namely, that the factors which determine the pattern of development in the blastema have *field character.*

Regeneration as field activity.

The differentiation of a limb regenerate is directed and controlled by the *limb field* of the stump. As in ontogeny, the limb field is a property of the field district as a whole and should not be associated with any particular discrete group of elements. In fact, any sufficiently representative part of a limb may be found endowed with a whole limb field. Any slice of limb transplanted to a heterotopic position can regenerate the missing distal portion;[1] the orientation of these regenerates coincides with the orientation of the transplanted stump, and if the latter has been rotated on the body, the regenerate is likewise distorted.[2] These cases prove that *the body as a whole exerts no influence upon the character and orientation of a regenerate;* only the field of the injured organ itself is responsible for the qualitative determination of the regenerative process. When a lateral half of a limb is removed and the distal part of the remaining half is amputated, the blastema which forms at the latter cut produces a *harmonious limb,* in spite of the fact that the base of the regenerate contains only *half* the cross section of a normal limb[1] (Fig. 105). Obviously, lengthwise splitting of a limb leaves each half in possession of a total limb field, and this totality finds expression in the harmonious organization of the newly forming blastema.

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[1] *de Giorgi*, 1924.

[2] *Weiss*, 1924.

The *limb field* extends over a certain radius around the limb base; this is evidenced by the fact that the regeneration of a new limb can be provoked from the body wall in the shoulder region even without amputating the original limb.[2] One is reminded of the wide extension of the limb-forming territory in the larva (p. 367). However, just as in ontogeny the radical removal of an emancipated local field district entails the permanent loss of the corresponding organ (p. 321), so the complete extirpation of the district of a developed organ precludes its regeneration.[3] Complete ablation of the shoulder region, including the free limb, is followed merely by the formation of a scar, and the region

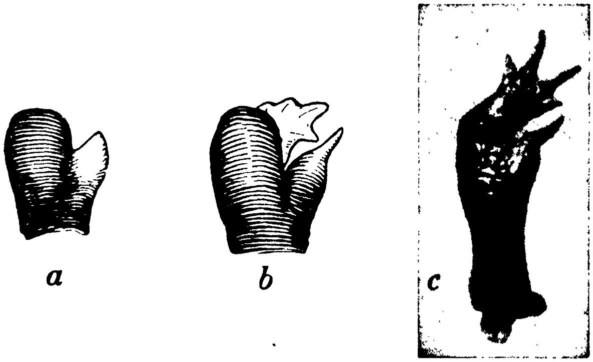

FIG. 105. Regeneration of a whole limb from a halved limb stump. (From *P. Weiss,* 1926) After amputation of the wrist and lengthwise splitting of the forearm a complete hand regenerated on one half and an atypical cone on the other.

a, 32 days after the operation.
b, 55 days after the operation.
c, End product.

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[1] *P. Weiss,* 1926a.

[2] *Locatelli,* 1924.

[3] *Guyénot,* 1927b.

remains limbless.[1] Similarly, *eye* regeneration occurs only if some remnant of the bulb has been left behind in the orbit, whereas complete enucleation of the bulb apparently removes the whole eye field district and thus precludes regeneration.[2] For similar reasons, a completely exarticulated *bone* is not replaced [3] (see example 96), whereas bone regeneration occurs if a fragment of old bone is left behind; thus, *regeneration of bone alone* in an old limb is a process of different order from that of *differentiation of bone within a limb regenerate.* The limits of the *jaw* [4] and *tail* [5] field districts in the adult have likewise been established by varying the levels of ablation until regeneration failed to occur.

The post-embryonic subsidence of regeneration fields.

It is reasonable to assume that these various fields are the active residues of the formerly much richer field equipment of the embryo. Only in certain organs, such as the limb, eye, tail, etc., do fields persist far beyond the embryonic stage. In the other organs they are dissipated. We know neither the reason nor the physiological basis for these differences; if we knew, we would probably have a more tangible conception of the nature of fields. It is very remarkable, for instance, that organ fields survive into the *adult* stage in the *tailed* amphibians, while in the *tailless* forms they subside at the end of the *larval* stage.[6] In the anuran tadpole the capacity for limb regeneration ceases at a certain phase of metamorphosis. That this loss is due to some change within the limb rather than within the body, is proved by the following experiments.

Example 99.[7]

Limbs were cross transplanted between larvae of tailless and tailed forms. At the critical time of metamorphosis parts of the grafts were amputated and tested for regenerative capacity.

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[1] *Kurz*, 1922.

[2] *Schaxel*, 1921.

[3] *Wendelstadt*, 1904.

[4] *Valette*, 1929.

[5] *Schotté*, 1926.

[6] *Barfurth*, 1895.

[7] *Guyénot*, 1927a.

The results were clear cut. Even when transplanted to the body of a *salamander* of permanent regenerative capacity, the limb of a *frog* tadpole lost its regenerative capacity at the time of metamorphosis, while the control limbs of the host continued to regenerate. Conversely, *salamander* limbs regenerated in *frogs* whose own limbs had lost that faculty. In other words, there was *no relation between the regenerative power of the host body and that of the grafts.* This, incidentally, is additional proof that regeneration in higher forms is a strictly *local* affair and by no means an aimful reaction of the body as a whole.

Regenerative capacity hinges, first, on the presence of a residual local field and, second, the ability of the wound stimulus to mobilize a sufficient amount of indifferent cell material. The subsidence of regenerative capacity in an organ could, therefore, be due either to the *disappearance of the field* or to an *exhaustion of the cell sources* from which a blastema could be formed. An attempt to decide the alternative was made in the following experiment.

Example 100.[1]

Providing a non-regenerating limb with regeneration cells from a tail.

In the lizard an amputated *tail* regenerates promptly, while *limbs* are devoid of organized regenerative capacity; the amputation wound of a limb is simply covered with scar tissue and no further differentiation occurs.[2] If the failure of the limb to regenerate were merely due to its inability to supply an adequate number of cells for the formation of a blastema, this could be remedied by providing it experimentally with the necessary material; all one has to do is to amputate the tail, wait for the accumulation of an ample mass of regeneration cells, and then transplant this blastema to the limb stump. If the latter possesses an active limb field, limb regeneration ought to ensue. This, however, did not happen. Blastemas taken from the *tail* earlier than about two weeks after amputation *under-*

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[1] *P. Weiss*, 1930.

[2] A few cases of abortive growth in the place of an amputated limb have been reported; but there is never a semblance of limb formation (*Marcucci*, 1926; *Guyénot* and *Matthey*, 1928).

went no further differentiation when grafted upon the cut surface of a *limb*. Most of them were resorbed. Older blastemas under the same conditions produced *tail* regenerates (Fig. 106B), which indicates that the *general* conditions in the limb stump are by no means unfavorable for growth and differentiation. If, nevertheless, no limb regenerated, the failure must evidently be ascribed to a *lack of the specific field conditions* for the organization of limb tissues.

How indifferent are the cells of the blastema?

This experiment is not fully conclusive, however. One might object that even the young tail blastema, although not yet capable of self-differentiation into tail, might still not be indifferent enough to respond adequately to the exigencies of the limb field. This is a valid argument, and it raises the question as to how indifferent the regeneration blastema is in reality.

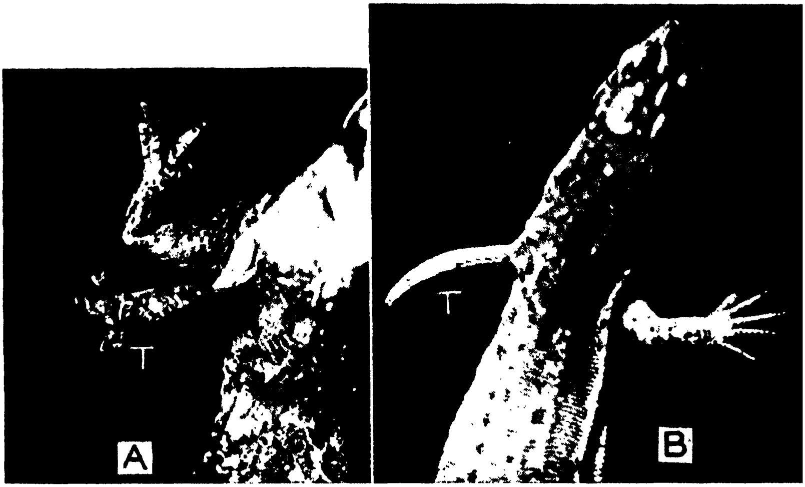

FIG. 106. Differentiation of regeneration blastemas of the tail after transplantation to the site of the fore limb.

A, In the newt, *Triton cristatus*, an accessory limb (T) has developed after transplantation of a tail blastema of 6 days.

B, In the lizard, *Lacerta agilis*, a tail (T) has developed from a transplanted tail blastema of 15 days.

On an earlier occasion (example 89), we have described some observations suggesting that mesenchyme which has resided for some time in a definite location of the organism has acquired some distinctive growth potential which distinguishes it from mesenchymes of other locations. Might this not be an indication of some profound differences which, in the last analysis, might make the mesenchyme of a tail unfit for utilization in the formation of a limb? An attempt to decide the question has been made in amphibians in which both tail and limb are good regenerators.

Example 101.[1]

Tail blastema grafted into the limb field.

The *tail* of a newt was amputated and the accumulation of the regeneration blastema over the cut surface was awaited. Blastemas of varying ages were then transplanted into a wound made near the base of the *fore limb*. In view of the indispensability of nerve supply for the progress of regeneration (see p. 446), some limb nerve was also deviated toward the graft. Just as in the lizard, tail blastemas which had been allowed to stay in the original tail territory for a certain critical period, continued to develop *tail-like* features at the limb site. Although the general tail character of these regenerates was unmistakable, they never developed either the size or the perfection of ordinary tails. This is another illustration of the fact that the determination of an organ, in this case of a tail regenerate, is not of all-or-none character (p. 415); evidently, at the time of transplantation, only some of the tail features had been fixed in the transplanted material while others were not yet determined. *Younger tail blastemas,* transplanted before the critical age, however, *gave rise to clearly identifiable limb regenerates* (Fig. 106A).

If these limb regenerates have actually developed from the transplanted tail material itself, this would prove conclusively that the cells of the *tail* blastema are really indifferent to the extent that they can still be used in the building up of a *limb*. Of course, with the exception of the nervous system which is not of blastemal origin, the essential histological formations in a limb and a tail are very similar. They include musculature, skeleton, cutis, connective tissue, and joints. Only the morphological arrangement of these elements varies, depending on whether they differentiate in tail or limb fields (see p. 67). The production of a limb from a tail blastema cannot reveal, therefore, whether the cells of the blastema possess wider potencies than those inherent to ordinary mesenchyme. But, within these limits, at any rate, the cells seem to be fully responsive to the demands of the limb field. Similar results were obtained after exchanging blastemas between *fore limb* and *hind limb*,[1] and after grafting *tail* blastema into the *eye*.[2] In all these instances, blastemas removed from their original field during sufficiently early stages of formation were reported to have differentiated in conformity with the field of the new rather than the old location.

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[1] *P. Weiss*, 1927.

Inconclusiveness of the experiments.

However, a serious objection can be raised against this version of the experiments. One can argue that possibly none of the regenerates have really developed from the *grafted* material: the transplants might have been *resorbed* and *gradually replaced by cells indigenous to the locality*. Since none of the experiments have thus far employed methods that would invalidate this objection, the question as to whether different blastemas are really interchangeable during the early stages of regeneration cannot be considered settled. Tagging of the transplanted cells, such as is done in embryonic transplants (vital staining, heteroplastic grafts, etc.; see page 129 f.) would clarify the situation. Pending crucial tests, an attitude of reserve must be maintained toward claims of unlimited potencies in regeneration cells of vertebrates.

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[1] *Milojević*, 1924.

[2] *Schotté and Hummel*, 1939.

Summary: The epigenetic character of regeneration.

The continued study of regeneration phenomena promises to open many fruitful avenues of approach toward the solution of such basic problems of development as growth, differentiation, morphogenesis, and secondary adjustments between parts and the rest of the organism. The fact that regeneration in a higher animal involves hundreds or thousands of cells, puts the collective character of developmental phenomena into sharper relief than do the early ontogenetic phases during which the role of the individual cell elements is frequently unduly accentuated owing to the incomparably smaller numbers present. Any such ideas as were current in the earlier days of experimental embryology, suggesting that the differentiation of parts and the spatial organization of an organism may be the outcome of stereotyped sequences of cell division during cleavage, defeat themselves in view of the phenomena of regeneration which occur on such a large scale that the direction in which an individual cell divides or moves is of as little consequence for the result as the behavior of an individual soldier is for the outcome of a war. Even if one could be satisfied by the explanation which the old mosaic theory had to offer for ontogenetic development, namely, that the essential parts of the organism are preformed as such in the undeveloped germ, the explanatory value of such a view would fade in the light of the facts of regeneration, where, as we have seen, *differentiation of parts occurs definitely de novo within an originally homogeneous and equipotential mass of cells.* Here the basic problem of development — how parts which have not been so before become different from one another — rises again in its full import, and if it were for no other reasons, an *epigenetic* view of development would have to be postulated on the strength of regeneration phenomena alone.

MALFORMATIONS

The individual variability of ontogeny.

We have now accompanied the individual up to the adult stage. At this stage, the developmental processes have reached their final realization. But in no two cases have the final products turned out to be exactly identical. Individuals vary according to both initial inherited inequalities and random variations of the external circumstances attending development. Averaging the individual variations among all members of a species, one obtains an abstract ideal "type" around which the *majority* of individuals is grouped within fairly close range. This range, accommodating the fluctuations of the majority of individuals in a group, may be called the "*norm.*" Individuals whose deviations from the ideal type fall within the norm, are classified as *normal* (see p. 25). Individuals whose deviations from the common type exceed this limit, are called *abnormal,* and if the abnormality is primarily one of form, one speaks of *malformations.* The science dealing with these aberrations is known as *teratology;* it would be to the point to designate it as *pathological embryology.*

As one can see, the difference between normal and abnormal development is essentially of *statistical* nature. They differ neither in principle nor in character. On no account is it permissible to treat malformations as a separate class of biological phenomena. Witness thereof is the continuous gradation from strictly normal cases over barely normal, then slightly abnormal, to plainly monstrous ones. Nowhere along the line is there a sharp division, and the rating is often merely a matter of taste or convention. We shall not attempt here to bring even a brief survey of teratological phenomena, nor shall we go into the usual classification of malformations based largely on descriptive and superficial aspects. We only propose to outline how all deformities are amenable to a natural and consistent explanation on the basis of the dynamic view of development set forth in the preceding sections.

The compound character of individual variation.

One thing that will have become clear by now is the strictly dual aspect of each developmental manifestation; dual in that, first, each living part possesses definite *intrinsic* potencies, or potentials, such as for growth, differentiation, reactivity, etc.; and, second, that the realization of these potentials is conditional on factors *extrinsic* to the part, partly specific, partly unspecific, partly furnished by the rest of the organism — the internal environment — partly by the external environment in which the organism lives. Every single character of an organism, with the exception of such primordial properties as nuclear size, biochemical species differential, and the like (see pp. 131, 310), is invariably the result of a chain of processes in which *potentials are gradually led to realization in reaction to, and counteraction with, a varying set of circumstantials.* Both potentials and circumstantials fluctuate independently; the interindividual variation is, therefore, of *compound origin*, and so are the excesses of variation which we call abnormal.

Abnormalities can arise from either abnormal potentials or abnormal circumstantials, or both. On the other hand, abnormal circumstantials and abnormal potentials may also be mutually corrective, as can be best illustrated in growth (cf. Fig. 3): an individual of *excessive* growth potential can still keep within normal bounds if the circumstantials (temperature, food, etc.) happen to have *subnormal* values; conversely, an individual starting with a *subnormal* growth potential may reach normal size if faced with *supernormal* circumstantials.

Abnormal growth.

From the standpoint of embryonic dynamics, there is no such thing as abnormal *size*. There can be only abnormalities of *size-controlling factors*, and since, as has been shown before (p. 421 f.), there is a considerable variety of such factors in operation, a given abnormality of final size may be the result of any one of a number of causes. Excessive variations of the initial amount of protoplasm (p. 301), of growth rate (p. 45), of food (p. 20), of the duration of the growth period (p. 436), to name only a few, may entail abnormal dimensions of the developed body. The fluctuations of any two of the cited component processes may be subtractive as well as cumulative.

The same considerations as here outlined for the whole organism then apply to the individual organs. As has been stated above (pp. 421, 437), the final size of each organ depends on a variety of factors of which initial mass of cells reclaimed by the organ field (Fig. 95), intrinsic growth rate ("partition coefficient") (p. 432), spatial limitations, nutrient level, hormone influences (p. 433), activity (p. 447), are but a few selected ones. Abnormal levels of any one of these numerous properties and activities can upset the normal proportions of the body by reducing or enlarging the size of an organ relative to the others. Although later adjustments (see p. 435), especially "functional adaptation," may correct some incongruities resulting from the disruption of the normal growth balance, the scope of such regulations is strictly limited, and beyond its range abnormalities of the growth processes become fixed as permanent malformations.

Abnormal differentiation.

So far we have spoken only of *growth.* If we widen the picture so as to take in *differentiation* and *organization,* the complexity increases enormously. Differentiation is a highly composite act. There is no need of reiterating this point in view of the emphasis placed on it in the past discussion. We know the many prerequisites of differentiation: specific differentiation potency in the cell, activating stimuli, energy supply, directive field factors, adequate milieu, inductive interactions — all these must be present to yield an individual of some degree of organization, and must be present in definite patterns, dosage, and timing, in order to yield a *normal* individual typical of its species. And again, just as in growth, excessive fluctuations of any of the quoted prerequisites can throw the whole developing system off balance and produce freakish individuals.

Once more we may compare the development of an organism to an utterly complex stage play engaging numerous actors, providing for major and minor roles. There is a preliminary phase in which roles are assigned, cues fixed, appearances timed and the stage set; then there follows the actual performance. The orderliness of the performance depends equally on the correctness of the script and on the accuracy with which it is put into effect by the actors. The play is spoiled when a major actor either drops out, makes an untimely entrance, leaves at the wrong moment, talks out of turn, overshouts the others, or otherwise falls short of harmonious coöperation. In development it is the same story: *component events whose timing or dosing is out of tune with the general plan disturb, and often even break up, the whole performance.*

Suppression of parts.

If a developing part drops out in the preliminary phase, prior to final determination, other parts may substitute, and the roles are redistributed. No permanent defects result. Accidental *splitting* of the company of cells in the germ at this time may cause the staging of two performances instead of one: *twinning.*[1] This may be due to mechanical (see p. 326 f.) or dynamic isolation of the two groups. When the same accident happens to an organ rudiment in its equipotential state, *reduplications* of various degrees may be produced. This appears to be one of the causes of *hyperdactylism* (supernumerary digits)[2] in limbs.

If a part drops out after a mosaic state has been attained, the loss is permanent. Partial defects or total absence of limbs in mammals at birth (example 6) have been ascribed to local amputation of determined parts of the limb buds by amniotic folds or similar accidents.[1] Differential susceptibility to general toxic agents (p. 159) likewise accounts for the suppression of localized parts. Which parts are suppressed depends on the developmental stage of the organism at the time when the agent strikes, since susceptibility, as we have said (p. 376), varies with the degree of activity (metabolism?), and the centers of developmental activity shift from stage to stage. Thus, temporary depressions of living conditions may produce permanent defects by harming those regions of the embryo which happen to be the most sensitive at the time of the contingency.

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[1] *Newman,* 1923.

[2] See, for instance, *Bagg,* 1929.

Secondary effects of the suppression of parts.

With the elimination of an organ all its subordinate structures are, of course, affected, too (see example 6). In further consequence, the local suppression of a part permits neighboring structures which are normally held apart to merge. This is the case in *cyclopia*,[2] (p. 147) where the suppression of the rostral block of tissue which ordinarily separates the eyes leads to their complete median fusion (Fig. 107). Depending on the strength of the harmful factor, the time and duration of its operation, and the resistance of the organism, the median defect varies in extent. Accordingly, various degrees of convergence and fusion of lateral organs can be obtained. Genetic studies have been most helpful in establishing such graded series of malformations produced by the aberration of a single developmental condition[3] (Fig. 108). It is instructive to keep this situation in mind as evidence that not only may *the same type of malformation arise in various ways*, but *various kinds of malformations may also be caused by the same disturbance.*

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[1] More recently the assumption of intrauterine amputations by amniotic folds has been discredited, which leaves these cases without reasonable interpretation; one could think of secondary resorption due to accidental occlusion of the blood vessels and stoppage of nutrient flow.

[2] *Adelmann*, 1936. [3] *Wright and Wagner*, 1934.

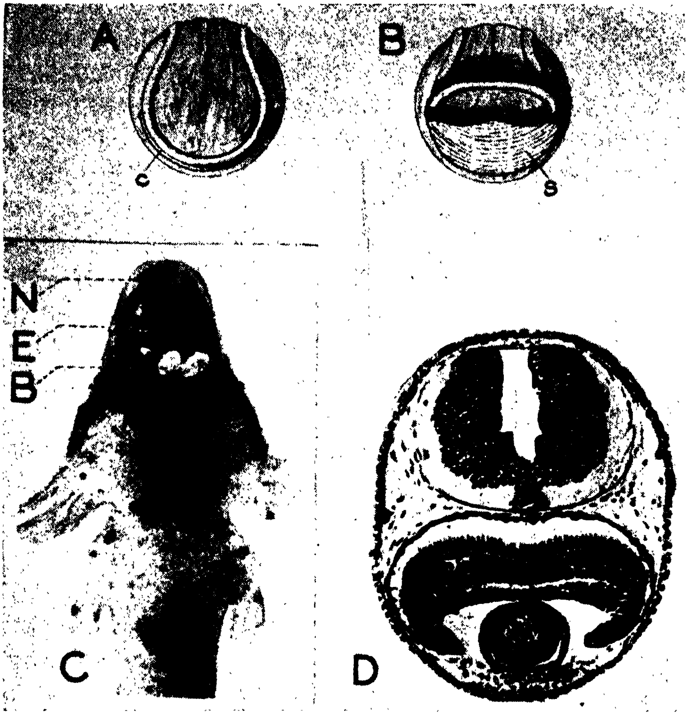

FIG. 107. Cyclopia in the newt, produced by mechanical defect. (From *Mangold*, 1931) Excision of the mesodermal substratum of the anterior part of the medullary plate in the early neurula stage has entailed median fusion of the eyes into a single ventral eye.

A, Neurula prepared for operation by cut (c) around the anterior border of the medullary plate.

B, Anterior part of medullary plate is lifted. The mesodermal substratum (s), thus exposed, is then excised and the medullary flap is put back in position.

C, Ventral view of cyclopean larva developed from an operated germ. N, Joined nostrils. E, Median single eye. B, Balancers. × 14.

D, Cross section through the head of C, showing the ventral eye with single median lens. × 42.

This case imitates the chemically produced cyclopia of the squid (Fig. 21) and the inherited cyclopia of the guinea pig (Fig. 108, fourth row).

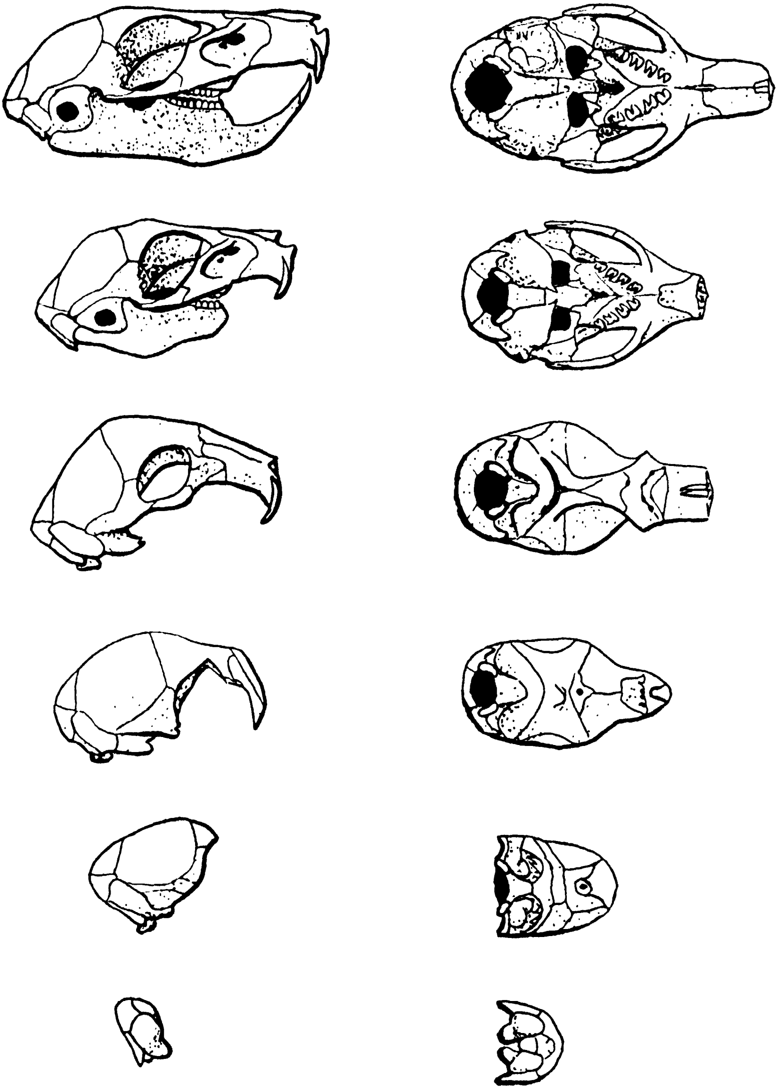

Fig. 108. Progressive reduction of median parts of the head in inbred strains of guinea pigs. (After *Wright and Wagner*)

Left column: Lateral view of whole skull. Right column: Ventral view of base of skull. Top row: Normal guinea pig. Second to bottom row: Increasing extent of defect (*otocephaly*).

When a local defect suppresses a vital organ or some vital function, it becomes *lethal.* Abnormally low viability, therefore, is not necessarily based on a common deficiency of all cells but can be due to localized causes (nervous paralysis, heart failure, local autolysis with subsequent intoxication, digestive failure of the intestine, and the like). This fact is illustrated by the behavior of cells from embryos of genetically lethal constitution: embryos of this kind do not survive a certain critical phase of development; however, cells removed from the body before death and explanted in tissue culture, carry on and multiply[1] (cf. also example 12).

Abnormal timing and dosing of developmental events.

That the correct *timing* of the interactions of parts is as indispensable for harmonious development as is their proper spacing has been mentioned repeatedly (pp. 104, 319, 558). The adverse effects of untimely hormone actions may serve as illustrations (pp. 71, 436).[2] And a similar thought finally applies to the *dosing* of embryogenetic factors. Wherever the intensity of an action determines the strength of its developmental effect, the overdosing of one such factor can unbalance the whole subsequent course of development.

Hereditary abnormalities.

Some abnormalities are *inherited;* that is to say, in the strains so affected an aberration from the normal developmental pattern of the species has become established as the norm, and what in regular strains occurs only as a sporadic accident has come to be of systematic recurrence. Twinning, hyperdactylism, cyclopia, taillessness, and many other such characters can result from occasional *phaenotypic* modification of an *individual germ* or from a *genotypic* revolution (mutation) of the developmental potentials of the whole *strain.*

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[1] *Ephrussi,* 1935.

[2] *Goldschmidt* (1938) has presented a full discussion of the consequences of chronological disturbances of development.

Example 102.

> Certain strains of mice show hereditary defects of their fore limbs.[1] An analysis of their embryonic history[2] revealed the actual cause of the disturbance. Apparently the cells producing cerebro-spinal fluid in this strain overdo their job and exude more liquid than brain ventricles and spinal canal can hold. The excess flows out and follows beneath the skin the nearest valley in the surface topography of the embryo, which at that stage happens to be a groove down the neck into the fore limb. Thus, by accident — but a systematic accident, indeed — the drop of liquid gets to the limb bud where it is stopped in the blind end. *It is the pressure of this foreign substance then which interferes with the further differentiation of the limb and causes the defects of its morphology.*

The mechanical disturbance of limb development here corresponds to all purposes to those artificial injuries through which an experimenter can provoke similar limb defects at will. Little does it matter to the result that, in the present case, the embryo itself plays the role of the experimenter. The case of *cyclopia* provides another example. The immediate cause of the cyclopic fusion of the eyes is their failure to become separated by a wedge of median neural plate and mesoderm (see above). But it is irrelevant, so far as the ultimate effect is concerned, whether this failure is based on a mechanical removal (Fig. 107), chemical suppression (Fig. 21), or intrinsic hereditary weakness (Fig. 108), of this median strip. Many additional instances could be outlined. They include the so-called "*phaenocopies,*"[3] which means cases in which genotypic (mutational) aberrations can be imitated by phaenotypic actions. But a further discussion of these points would take us too far into the domain of genetics, which is beyond the scope of our discussion.

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[1] *Little and Bagg,* 1924.

[2] *Bonnevie,* 1934.

[3] *Goldschmidt,* 1938; *Kühn,* 1936.

Malformations as deviations from the standard course of development. In general, *morphogenetic* aberrations, excesses as well as deficiencies, can be traced to individual excursions beyond the normal range of variability of some of the innumerable conditions controlling the regular dosing, timing, and spacing of developmental activities. *Histogenetic* aberrations (albinism, melanism, haemophilia, etc.) are more commonly referable to deficiencies in the differentiation potencies of the cells. The distinction is by no means sharp since many phases of the cell response based on differentiation potency (e.g., the production of hormones) assume morphogenetic significance.

We may conclude by saying that malformations are *merely modifications of the standard developmental patterns, rather than developmental innovations.* They put into sharp relief the fundamental tenets which observation of normal embryogenesis and analysis of experimentally modified embryogenesis have taught us: *the multiplicity of developmental agents; the harmony of their coöperation; the preformation of the differentiation potencies of the cell; the selective activation of these potencies by epigenetic actions of fields; the universality of the principle of progressive determination; and the purely statistical character of "normality."*

PART FOUR

THE DEVELOPMENT OF THE NERVOUS SYSTEM (NEUROGENESIS)

A DESCRIPTION OF THE NERVOUS SYSTEM

The complexity of the nervous system.

It does not require very intimate study to become aware of the fact that of all the highly complex institutions of the body the adult nervous system has the privilege of being the most intricate one. Nerve anatomy and physiology struggle perpetually with the bewildering complexity of its devices, structural and functional. However, while to anatomists and physiologists only the finished machine is of concern, the embryologist is called upon to explain how all this complexity has come about. Fortunately, his task is not quite so forbidding as it at first appears. For, in neurogenesis, as in all ontogeny, complex products are built up by simpler and more elementary steps, each one singly accessible to methods of study which it would seem futile even to attempt to apply to their totality. One is reminded of the subterranean network of telephone cables, water and gas mains, electric power lines, sewers, and subways honeycombing a modern city; one can hardly conceive of any such intricate and elaborate system as ever being set up in final perfection by one single step. It is the thousands of additions and improvements added in the course of time that make the system look so complicated in the end. Similarly, the nervous system does not assume its final shape and organization in one stroke but acquires it gradually in a long chain of progressive modifications.

The morphological aspect.

Morphologically, the central nervous system appears as a sculptured mass of typical outlines and proportions. There are compact bodies of typical size and regular distribution, the ganglia (cranial, spinal, and sympathetic), and there is the typically shaped continuous mass of brain and spinal cord, surrounding a lumen, the central canal, with its anterior widenings, the brain ventricles. The subdivision of the brain into different portions is the same in all normal individuals of a species. Bulges, folds and grooves of the brain wall appear in typical and constant locations. Internal differences, corresponding to the external molding, become evident even to gross inspection: well circumscribed areas can be recognized which differ from one another by their characteristic form, consistency, grouping of cells, staining properties. Again, these foci or "nuclei" are found in constant positions, and so are the various fiber tracts interconnecting them.

The peripheral nerves connecting the central nervous system with the non-nervous receptors (sense organs) and effectors (muscles, glands, chromatophores), are likewise arranged in a rather definite pattern; their number, individual size, distribution, branching, anastomosing (plexus formation), course and final connections, although varying somewhat in detail, still follow a certain common norm.

This first crude description raises numerous questions concerning the morphogenesis of the nervous system. What makes the central system hollow, what keeps it hollow, what causes the inequalities in its wall, the appearance of folds and swellings and of localized nuclei, what determines the distribution of white and gray matter and their typical relative proportions? What, finally, is responsible for the fact that intracentral nerve tracts as well as peripheral nerves are laid down in definite sizes, sites, and patterns?

The histological aspect.

Histologically, the nervous system presents us with a variety of cellular differentiations exceeding that of any other type of organs. Although its cells can be classed into two main groups, glia cells and nerve cells proper, neither of these groups is in itself uniform. Among the nerve cells proper, which are the elements of the truly nervous function of conduction and integration of excitations, many distinct types can be recognized differing considerably in regard to size, shape, and inclusions of the cell body as well as in regard to number, length, and mode of branching of their axones and dendrites. Some are very abundant and rather ubiquitous, while others are strictly confined to certain locations and sometimes are very scarce, as, for instance, Mauthner's cell, a giant form found in fishes and amphibians, of which there is only one pair present in each individual.

The neuroglia, whose function is presumably one of support, protection and trophic assistance to the nerve cells, also displays a variety of cell forms. Inside of the brain and spinal cord, it forms a continuous matrix in which the nerve cells lie embedded, while in the peripheral nerves and ganglia, it is represented by long slender cells (cells of *Schwann*) enveloping each axon with a layer of nonconducting protoplasm. Both in the centers and in the periphery, the presence of glia cells seems also to be prerequisite for the formation of myelin sheaths around the nerve fibers. The possession of a myelin sheath is a privilege of certain types of nerve fibers only, and, again, the kinds and numbers thus endowed are strictly circumscribed.

These histological facts add more questions to our list. What causes the cells to differentiate along those various lines and what makes them accumulate, associate and interconnect in certain places, or scatter more diffusely, as the case may be? What induces them to produce axones and dendrites in definite numbers, in a definite order, of definite sizes, and with definite properties, such as the capacity or incapacity to form myelin? What, then, causes the axones and dendrites to be oriented in definite directions, what controls the extent of their arborization, the numbers in which they are assembled into nerves, and the fact as well as the details of their termination in or upon other cells, central or peripheral?

The functional aspect.

The *functional* picture is even more intricate than the morphological and histological diversity would have allowed us to predict. Not only do most of the observed visible differences among nerve cells and fibers reflect functional peculiarities, but the functional differentiation goes far beyond the point of morphological discriminability. Nervous elements which under the microscope appear alike have often been revealed to differ significantly in their functional properties, e.g., their excitability, fatigability, affinity to drugs, and the like. The development of these functional characters certainly deserves as much attention as that of the more conspicuous morphological characters.

Finally, it is the duty of the embryologist to study the development of those highest nervous functions which manifest themselves in the behavior of an animal and in the mental activities of man.

This list indicates roughly the task confronting the student of neurogenesis. It refers to the most fundamental issues only; of these only a small fraction has been subject to analytical investigation, and of the ones that have, only a minority have thus far been brought anywhere near a solution. This leaves almost the whole way still to be gone. Some progress has nevertheless been made and the following pages may serve to illustrate it.

NEUROGENESIS

The Early Morphogenesis of the Nervous System

The shaping of the medullary plate.

The morphological development of the nervous system is initiated by the transformation of a clearly delineated median strip of the ectoderm into the *medullary plate.*[1] The outline of this strip has roughly the shape of a spoon (see Fig. 109A). This shape anticipates the later morphological subdivision of the central nervous system into brain and

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[1] The following description is based on the situation as it occurs in amphibians; other vertebrates fall in line so far as the essential features are concerned, but deviate in details.

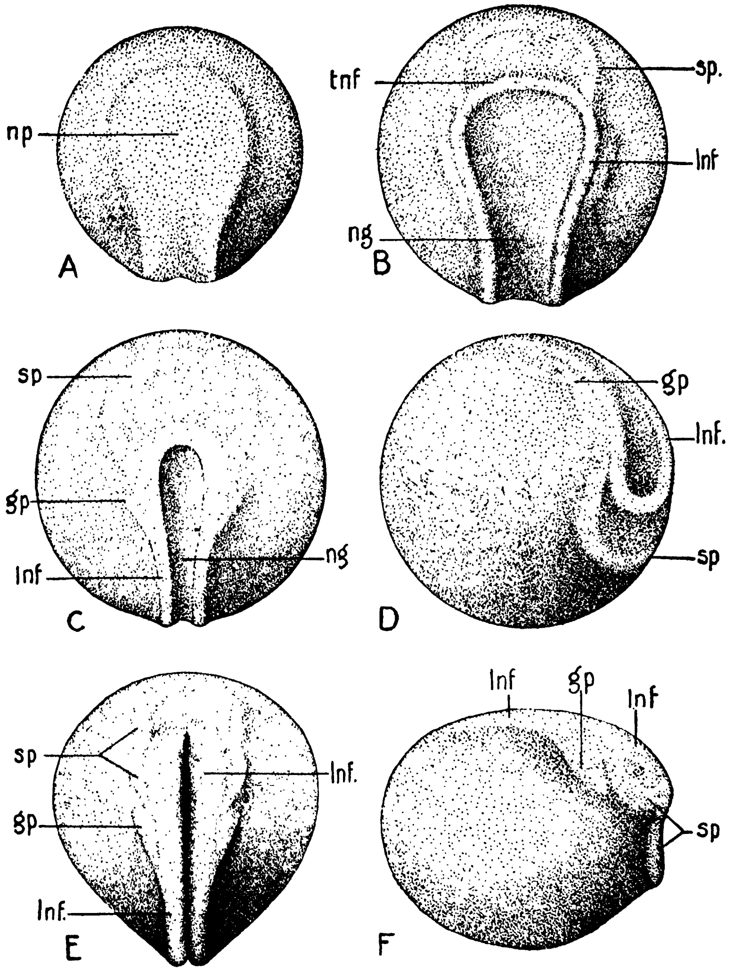

Fig. 109. Transformation of the medullary plate into the neural tube in the frog. (From *McEwen*)

A, B, C, E, Successive stages of the closure of the tube.

D, F, Lateral views of C and E.

np, Medullary plate. ***ng***, Neural groove. ***lnf***, ***tnf***, Medullary (neural) folds.

spinal cord. Visualize a spoon in which the edges all around, including those of the handle, are bent upwards until they meet and fuse enclosing a cavity in the interior; the spoon thus assumes the shape of a retort. This is precisely what happens to the medullary plate. Its edges, called medullary folds, rise and finally fuse dorsally to establish a retort-shaped hollow structure which represents in its wider vesicular part the later brain, and in its narrower tubular part the later spinal cord (Fig. 109). The delineation of the medullary plate has the same importance for the primitive shaping of the central nervous system that the correct cutting out of material has for the fit of a garment. We are thus referred back to the factors determining shape, extent, and proportions of the medullary plate.

It was described above (p. 345) that dorsal ectoderm receives its impulse toward nervous development from the invaginating mesodermal substratum through specific actions of the latter. It seems that these activities, besides determining the quality of nervous development, are also responsible for the outlining of the area which is to take part in it.

Example 103.[1]

Mesoderm affecting the size of the plate.

In an amphibian gastrula a lateral defect was produced in the invaginating chorda-mesoderm plate, eliminating thus on one side part of the prospective substratum of the nervous system. Under these conditions, *the medullary plate turned out to be asymmetrical, undersized on the side of mesodermal reduction,* and this without the operation having caused any direct injury to the presumptive medullary ectoderm (Fig. 110).

Evidently, then, the mesodermal substratum has an important bearing on the amount, and possibly also the outline, of ectoderm allocated for the medullary plate, and this, in turn, as we have just seen, is of significance for the gross shape of the early central nervous system.

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[1] *Lehmann,* 1928.

Changes in cell shape.

The earliest distinctive character of the plate, as compared with the surrounding ectoderm, is the markedly increased height of its cells, which gives it greater thickness and mechanical rigidity. This heightening of the cells could be due either to passive deformation produced by lateral compression, or to an active change within the cells themselves. The evidence points toward the change being active; in many transplantation and explantation experiments cells have assumed the general heightened appearance and shape of medullary cells in situations obviously free from lateral pressure. We conclude, therefore, that these cells possess some intrinsic tendency to grow in height. It is important, however, to note that in the embryo, as the cellular elongation causes the plate to become thicker and firmer, there is also a reciprocal influence by which the mechanical conditions of the plate as a whole reflect upon the shape of the constituent cells; especially during the subsequent bending of the plate do the individual cells suffer marked passive deformations. The situation reminds one of that occurring when people get crushed in a crowd which they have formed themselves.

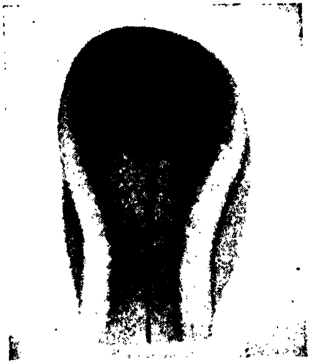

FIG. 110. Asymmetrical development of neural plate (marked weakness of right side) following lateral injury to the mesodermal substratum. (From *Lehmann*, 1928) × 22.

The formation of the neural tube.

The medullary plate gains in height at the expense of its surface extension. This decrease of surface area is compensated by a corresponding expansion of non-neural ectoderm toward the dorsal side of the germ. Then the plate rolls up to form the neural tube; thereby more material disappears from the exterior, accompanied by continued dorsad expansion of adjoining surface ectoderm. So the medullary and the extra-medullary districts move hand in hand. This raises the question as to which one is the active partner in the process. Does the medullary plate bend actively and thus drag the extra-medullary ectoderm upward; or does an actively expanding ectoderm push dorsad, thus folding up an inert mass of neural material? In one of the first experiments ever done in experimental embryology the question was decided in favor of the medullary plate; at least, it was shown that the medullary plate when isolated could bend into a tube without external assistance.[1] The folding of the plate is, therefore, an *autonomous* act.

The mechanism of the folding process.

As for the mechanics of the process, our knowledge is very fragmentary. Neural tube formation bears a certain resemblance to the invagination of the entoderm in an echinoderm gastrula. In both cases a circumscribed region of the convex surface epithelium of the germ changes its curvature and turns rapidly concave. A model thought to give a fair representation of gastrula invagination was described above (Fig. 29). It was based on the assumption that vegetative cells have a higher swelling capacity at their inner than at their outer poles. The bending of the neural plate is perhaps attributable to similar circumstances.

Example 104.[2]

Counts of cell numbers in the medullary plate before and after its transformation into the tube have yielded approximately identical figures. This proves that the bending of the plate is not due to asymmetrical cell proliferation, for apparently there is no appreciable proliferation during the bending. At the same time it has been noted, however, that *the volume of the plate increases to about three times its original value.* This increase is largely accounted for by the differential absorption of water by the plate which brings up the water content in the nervous system of an amphibian embryo to nearly 80%, as compared with less than 60% for the whole body.

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[1] *Roux*, 1895.

[2] *Glaser*, 1914.

Now if this water resorption should prove to be more intense on the inner surface of the plate, i.e., the one facing the body, than on the outer surface exposed to the medium, the autonomous bending would be explained on the same principle as gastrula invagination. Or possibly there is, at first, uniform swelling of the whole plate, followed secondarily by intense dehydration and shrinkage along the outer face; this would likewise lead to a concave shape. The data are too scarce to permit a final decision,[1] but they suggest that the forces bending the medullary plate result from differences in hydration between the convex and concave sides.

Adjuvant movements of non-neural ectoderm.

Although there can be no doubt but that a medullary plate possesses intrinsic means to form a neural tube by its own active power, it would be a mistake to conclude now that the expansion of the non-neural ectoderm must necessarily be passive. Not only does non-neural ectoderm when isolated from a gastrula and reared in salt solution manifest a strong tendency toward active expansion (see p. 417), but, to judge from certain experiments, it actually aids in the damming up of the medullary folds.

Example 105.[2]

By treating a frog gastrula with sugar solutions it is possible to stiffen the medullary plate to such an extent that its active folding ability is paralyzed. Under these conditions it is observed that the non-neural ectoderm continues to push toward the dorsal midline and succeeds in some cases in folding up the inert medullary plate about the median groove as a hinge, as one closes an open book. This demonstrates that there is a certain amount of *lateral pressure exerted upon the plate* by the adjoining non-neural ectoderm.

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[1] In a later paper *Glaser* has stressed the lack of evidence for *differential* water resorption in the plate (*Glaser*, 1916).

[2] *Giersberg*, 1924.

Taken in conjunction, experiments 2 and 3 lead to the conclusion that medullary plate and non-neural ectoderm are both taking an active, coöperative, part in the movements of neurulation. While capable of manifesting their individual abilities independently, they normally do team work by assisting and reinforcing each other (compare a similar situation regarding gastrulation movements, p. 417).

The filling of the neural canal.

These two factors seem to be supplemented somewhat later by a third one, namely, *secretory activity* inside the newly formed tube. After its closure the lumen of the medullary system is filled with a liquid. In the embryo this filling undoubtedly serves to provide turgor necessary in order to prevent the brain vesicles and neural tube from collapsing. What is the source of this liquid?

Example 106.[1]

Brain fragments, including the whole thickness of the brain wall of a chick embryo (8 days of incubation) were split parallel to the surface into an outer half and an inner half, the latter containing the cells which had lined the brain lumen (so-called *ependyma* cells). Both pieces were embedded side by side in a clot of blood plasma in tissue culture. Within a few days *vast amounts of liquid were found to have accumulated* around the originally *inner* fragments, whereas no liquid was present around the fragment which had come from the outer brain surface. Unmistakably the liquid is a product, direct or indirect, of the cells lining the brain ventricles, for only fragments including such cells have produced it.

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[1] *Weiss*, 1934a.

To judge from these results, the ependyma cells, which form the inner lining of the tube, are endowed with the capacity to secrete a liquid. The continued secretion of this liquid into the lumen of the closed tube creates a steady hydrostatic pressure from the inside which keeps the walls distended. Evidently this pressure has also a morphogenetic function in that it helps to widen the early brain vesicle whose greater volume capacity has been prepared by the greater dimensions of the anterior part of the medullary plate (see above). The pressure may also be a factor in the further molding of the brain, owing to regional differences in the thickness and rigidity of the brain wall: less resistant parts will bulge out and form lobes while the firmer parts will stay behind as constrictions. We lack, however, more definite information about these supposed effects of hydrostatic pressure on the configuration of the brain.

The source of further growth.

The most potent factor in the further shaping of the central nervous system is certainly the *differential growth rate* of different portions of its wall. After the formation of the neural tube the capacity to divide becomes essentially restricted to the cells bordering on its lumen. The reason for this is not known; either the more peripheral cells have proceeded too far with their differentiation to be still able to multiply — we remember the antagonism between differentiation and proliferation (p. 85) — or there is possibly some agent present in the central canal stimulating cell division in those cells directly bathed by it. Whatever the reason may be, these cells constitute the major source of new cells for the growth of the nervous system (Figs. 111, 113). The new cells remain either within the ependyma and produce further offspring or move peripherad and join the mass of nondividing cells (the so-called *mantle*) accumulating around the ependyma. The accumulation accounts for the spectacular enlargement of the walls of the neural tube and brain vesicles during the early embryonic period. This increase is not uniform, however; the tube does not receive an even padding in the manner of insulation wrapped around a pipe. There is more extensive growth in some directions than in others; also production of new cells is more active at certain levels than at others.

Subsequent development, therefore, hinges upon (1) the extent, distribution, and rate of cell proliferation in the ependyma, (2) the proportion of cells released from it, and (3) the direction in which these latter move and the places in which they settle. Although little is known about these points, some tentative idea can be obtained.

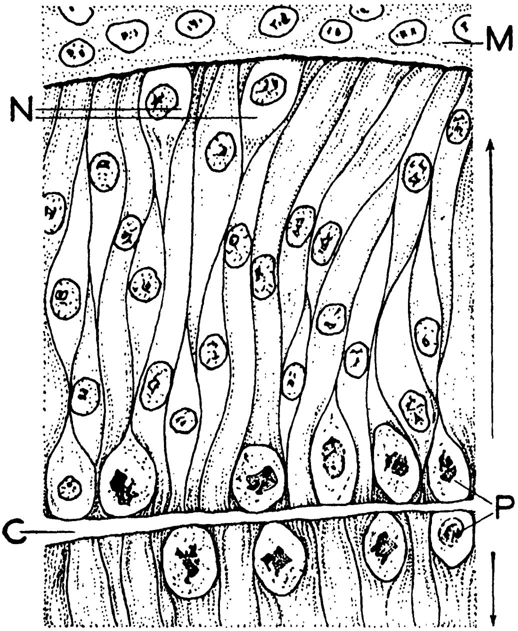

Fig. 111. Longitudinal section through the spinal cord of a chick embryo toward the second day of incubation (19 somites). (After *Lillie*) C, Central canal. P, Proliferating (germinative) cells. N, Neuroblasts settling in the mantle layer. M, Mesenchyme. Arrows indicate direction in which differentiating cells migrate off from the germinative layer.

The shape of the neural canal.

Let us first consider the *shape* of the ependyma, or what amounts to the same thing, of the central canal. In normal embryos it assumes the form of a slit extending dorsoventrally almost through the full height of the neural tube (Fig. 62). The elongate shape of the lumen enables a much larger group of cells to participate in its lining, and hence to proliferate, than would be possible if the canal were of the same width but cylindrical in shape (compare Fig. 112b and e). Therefore, the shape of the lumen is by no means irrelevant for the subsequent growth pattern. Now, is this slit-shaped arrangement of the ependyma an inherent character of the medullary development, or is it

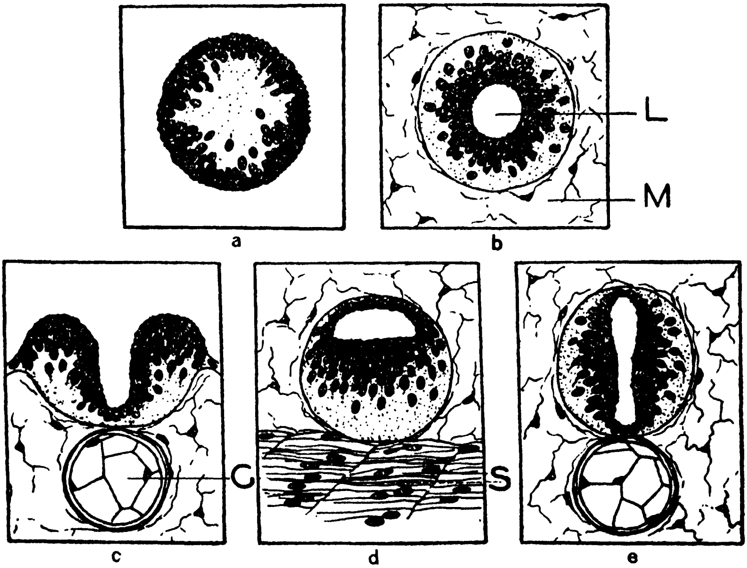

FIG. 112. Effect of extrinsic conditions on the morphology of the neural tube in amphibians. (From *Holtfreter*, 1934) For the normal shape of the tube, see Fig. 62.

a, Solid neural mass developed in explantation: nuclei of gray matter crowded near the surface; white matter in the interior.

b, Neural tube surrounded by mesenchyme: shape cylindrical with central lumen; nuclei massed at the inner (free) surface.

c, *Asyntaxia dorsalis* (failure of the tube to close). Thinning of the floor of the tube in contact with the notochord; gray matter along the free surface.

d, Neural tube underlain by musculature. Lumen eccentric at far side. White matter at near side (cf. Fig. 63D, m_g).

e, Neural tube underlain by notochord. Normal appearance. Slit-shaped lumen, oriented towards notochord.

L, Lumen. M, Mesenchyme. C, Notochord. S, Segmented musculature.

brought about by separate extrinsic factors? Certain observations point toward the latter view.

Example 107.

> The normal appearance of the central canal in an embryonic spinal cord of an amphibian is as in Figure 112e. Incidental to experiments in which the development of the notochord had been suppressed by lithium poisoning of the germ, it was noted that *the spinal cords of such specimens had a cylindrical instead of a slit-shaped lumen.*[1] Absence of the notochord for other reasons than chemical susceptibility was found to have the same effect (Fig. 112b).[2]

One must conclude, therefore, that some activity of the notochord itself, or at least connected with its presence, produces the slit-like arrangement of the ependyma. The two lateral rows of myotomes may play an adjuvant role. There is evidence that contact with musculature favors solid development of the neural tube, forcing the lumen to the far side (Fig. 112d). In the normal embryo in which two lateral masses of musculature flank the tube, the lumen, therefore, assumes an intermediate equilibrium position, coinciding with the plane of symmetry of the embryo. The origin of the more complicated outline of the lumen of the brain has not yet been studied.

Next to the shape of the ependyma, the *intensity* and *distribution* of its *mitotic activity* impress us as of pre-eminent bearing on the later configuration of brain and spinal cord. As we have said before, mitotic rate, especially in the brain, does not fluctuate at random, but follows a definite pattern of space and time. At a given stage, we find mitotic figures crowded in certain areas against a more sparsely dotted background. Such centers of more active proliferation flare up in definite locations, remain active for a certain period, and then subside, the activity shifting to some other region. The local mitotic epidemics of today will, of course, become the local enlargements of tomorrow. This can be seen from the following example.

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[1] *Lehmann*, 1926.

[2] *Holtfreter*, 1933c.

Example 108.[1]

In a cross section through the forebrain of a chick embryo on the second day of incubation mitoses are found in the distribution shown in Figure 113A. *There are distinct maxima of mitotic density in the lateral portions of the brain wall.* Correspondingly we observe more extensive *expansion* of the brain wall in these

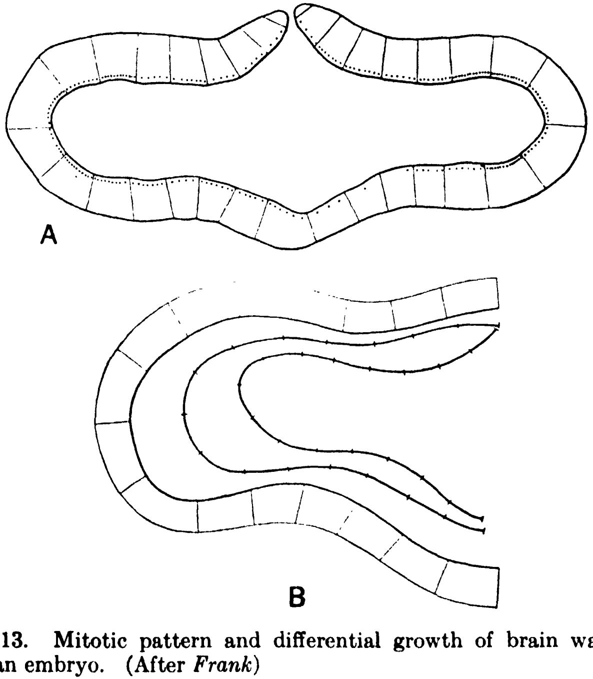

FIG. 113. Mitotic pattern and differential growth of brain wall in the amphibian embryo. (After *Frank*)

A, Distribution of mitoses. Composite picture of five consecutive cross sections through the closed medullary tube at the level of the forming eyes. The dots near the ventricular surface indicate the number of mitoses counted in each sector. Note the very marked crowding of mitotic cells in the lateral bends.

B, Three successive stages of the expansion of the embryonic brain. Only the inner (ventricular) surface of the left wall is shown. Note that greatest expansion corresponds to region of highest mitotic activity according to A.

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[1] *Frank*, 1925.

regions as compared with its total increase. Figure 113B, in which the inner outline of the brain is traced through three successive stages, shows this differential expansion. Incidentally, the lateral bulges in this example are the forerunners of the eye vesicles.

Mitotic density, in addition to its typical variation in the transverse directions, illustrated by this example, varies also in the longitudinal direction with peaks of activity appearing at definite levels. These patterns are typical and stable only for a given stage; they shift as the embryo grows older.[1] Figure 114 may serve as an example. Such definite inequalities in the growth rate of the wall are presumably

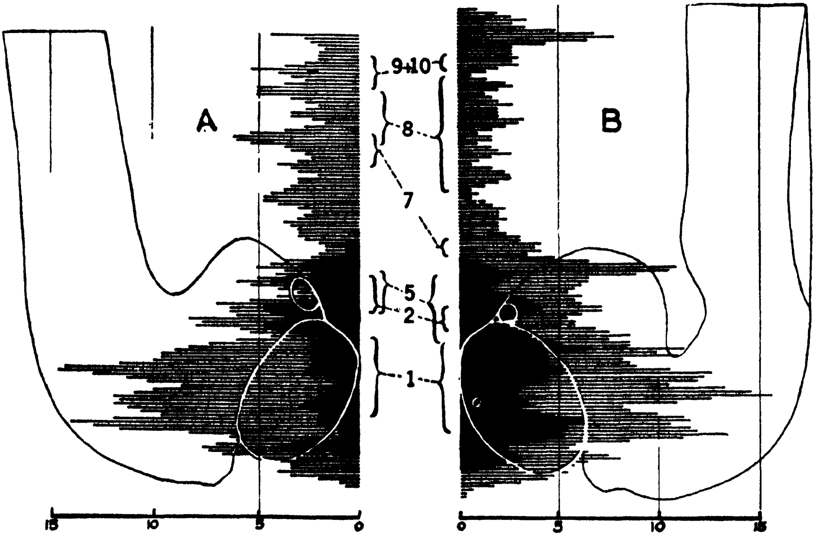

FIG. 114. Mitotic pattern of the brain of *Amblystoma*. (After *Burr*, 1932)
A, Earlier stage (31 after *Harrison*).
B, Later stage (35 after *Harrison*).
To facilitate comparison, the two stages are represented side by side in symmetrical arrangement. The outline of the brain is indicated. Horizontal lines express the number of mitotic figures contained in serial brain slices of 30μ each. Abscissa: Number of mitoses. Full black: Mitoses in forebrain and hypothalamus. Individual bars: Mitoses in the rest of the brain. Brackets indicate position and extent of sensory placodes. Note the change in the distribution of peaks from A to B.

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[1] *Coghill*, 1926, 1936; *Burr*, 1932.

the major instruments by which the primitive brain is shaped. Later cell migrations and fiber production become increasingly prominent, but the fundamental molding is undoubtedly a matter of the *growth patterns.* The mitotic pattern is of importance also in that it determines the course in which intracentral nerve tracts are laid down. For it has been stated that brain centers developing contemporaneously become connected by nerve fibers.[1] This would indicate that mitotic activity has an orienting influence on fiber growth; we shall return to this point on a later occasion (pp. 525, 535).

In view of the importance of the mitotic patterns it would be highly interesting to have more specific information concerning their origin. Unfortunately, the agents controlling the distribution of mitotic activity are still as obscure here as they are in regard to growth patterns in general. All we can say is that they have field character and in the case of the nervous system are attributes of the medullary field.

The direction of growth.

While mitotic rate determines the intensity of local growth, the direction in which the growing tissue expands is under the control of other factors. The type of growth illustrated in example 108 leads to surface expansion. In other instances, however, the result is mainly increase in the thickness of the wall. Obviously, the decision depends upon whether the newly formed cells stay in the inner surface or migrate off into the mantle layer. Both daughter cells arising from an ependymal mitosis have an even chance either to remain at the inner side and continue to divide or to move peripherad and differentiate. Since the former make for surface and lengthwise extension, while the latter produce an increase in thickness, it is clear that brain and cord can attain typical shape and proportions only if the ratio between cells retained in the proliferating layer and cells released from it is strictly controlled. Again, we are completely ignorant as to the manner in which this control is exerted, but one may infer from certain indications that *mechanical* factors are significantly involved. Stretch from faster growing structures in the surroundings, spatial limitations to surface expansion, and the hydrostatic pressure from the inside may all play a role in addition to possibly a host of other factors. It should prove rather easy to determine the effects of these agents by defect experiments.

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[1] *Coghill,* 1929.

The migration of cells.

Our next concern is the *course* along which the newly formed cells which are released from the ependyma shift into the mantle layer. Predominantly they migrate in a direction perpendicular to the surface of the ependyma. It seems that during the earlier stages this movement is governed by the transversal orientation of the elongated ependyma cells (see Fig. 111): the new cells glide along the older ones. Later, the whole matrix in which the cells lie embedded appears to possess a radial organization serving the cells as a guiding framework in the sense discussed above (p. 452) for the mesenchyme. The problem of the oriented migration of these cells thus resolves itself into the problem of the factors orienting their surroundings. Although no pertinent analysis has yet been accorded to these factors, they seem to be no different fundamentally from the ones outlined above as determining the patterns of colloidal ground-substances in general. In any case, there seem to be better reasons to suppose that the cells are conducted on their way by contact with the manifest structures of their surroundings than that they are directed by forces acting from a distance.

Here one must clearly distinguish between the fact that the cells move at all, and the fact that they follow a definite course. For the mobilization of the cells differences of electrical potential between the inner and outer surface of the neural tube may be responsible. Although the existence of such a difference has not yet been demonstrated, we know, at least, that if it existed it might have the supposed effect, for it has been observed that isolated neuroblasts in tissue culture when exposed to an electric field begin to move.[1]

The shaping of the brain wall.

Local growth and pressure from the inside, checked by the spatial limitations set up by the surrounding body, soon lead to the formation of folds and ridges in the brain wall. In this manner different portions become more sharply delimited. The production of nerve fibers by the differentiating nerve cells and the outgrowth of these fiber bundles modify the primitive form of the central nervous system still further.

Stratification of the brain wall.

Before long a new feature becomes noticeable: *stratification,* by which the brain wall is subdivided into several concentric layers of different aspect and, to judge from staining reactions and other indications, of quite different colloid-physical and chemical constitution. The appearance of strata in an originally homogeneous mass is not unusual in development. We do not know exactly how they arise, but some tentative idea can be advanced. When a mixture of chemical substances is allowed to diffuse into a jelly, the various components segregate according to their rates of diffusion; the faster ones forge ahead of the slower ones. A drop of diluted ink spreading on blotting paper gives a fair representation of the effect: a dark inner area formed by concentrated ink is set off rather sharply against a light halo of faster traveling water. If, furthermore, the penetrating substances react with other substances present in the substratum, layers of reaction products may arise which have the properties of membranes and bar further diffusion; such conditions inevitably lead to the appearance of distinct strata different in composition and consistency.

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[1] *Péterfi and Williams,* 1933. The results were not very striking, however. The most ardent exponent of the theory that the migration of neuroblasts is governed by differences of electrical potential is *Ariëns Kappers.* One knows that Protozoa exposed to an electric current in a liquid medium move toward one electrode (so-called galvanotaxis). It must be considered, however, that the medium in which embryonic neuroblasts move is apparently not liquid but gelatinous. Consequently, it seems unwarranted to place too much confidence in the Protozoan analogy.

Now, the matrix of the brain wall is undeniably such a jelly exposed to a chemical milieu whose composition is different on the inner and outer surfaces; the inner side especially with its mitotic and secretory activity is a potent source of chemical discharges. It seems not farfetched, therefore, to assume that the *lamination of the brain is primarily due to a differential impregnation of its colloidal ground-substances by chemicals* advancing into the depth of the wall for varying distances. Duplicating the physico-chemical stratification, the cell population will likewise segregate into corresponding strata and assume differential characters conforming to the various layers. Of course, once initiated, the relationship between cell and matrix becomes mutual again, as in all similar cases: the matrix affects the cells, and the cells act back on the matrix, so that at no time in the later course of events can either be credited with the final result to the exclusion of the other. It remains to be seen whether this conception of the origin of brain stratification is correct; in the meantime we may take it merely as a suggestion proving that a rational explanation is at least conceivable.

Segmentation of spinal ganglia.

While the brain and spinal cord have gone through the reported stages of development, the cells of the neural crest[1] have moved ventrad and now are found to be lodged between the ventro-lateral face of the neural tube and the dorsal mesoderm. At first continuous strands of cells, they become subdivided into segmental clusters, the *spinal ganglia;* these correspond to the segmental arrangement of the somites. Indication of an interdependence of the two structures has been obtained in the following experiments.

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[1] The neural crest is the cell mass lying wedged into the dorsal suture along which the neural tube has been closed. It is derived from cells along the edge of the former neural plate. While the tube closes, this cell mass escapes into the space between the convex aspect of the tube and the overlying ectoderm.

Example 109.[1]

Since the formation of somites proceeds in antero-posterior sequence, the younger somites from the more posterior levels are decidedly smaller than the older somites in more anterior segments. It is possible, therefore, to replace a given number of anterior somites by a larger number of posterior ones. When this was done in amphibian embryos, the spinal ganglia of the operated region were found to appear *in numbers corresponding to the numbers of somites actually present, and so spaced that there is one ganglion opposite each somite* (Fig. 115B). Extirpation of

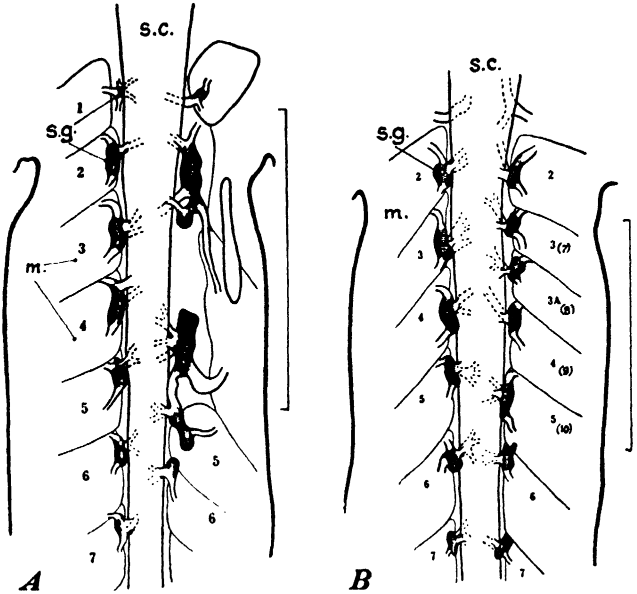

FIG. 115. Effect of myotomes on segmentation of spinal ganglia in *Amblystoma*. (After *Detwiler*)

A, Extirpation of the right somites, 2–4, in the tail bud stage has entailed lack of separation and irregular disposition of the ganglionic mass in the affected segments.

B, Substitution of four smaller somites, 7–10, for the three larger somites, 3–5, has resulted in the formation of four instead of three spinal ganglia in the operated region.

s.c., Spinal cord. s.g., Spinal ganglion. m, Somites. Bracket indicates extent of operation. × 13.

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[1] *Lehmann*, 1927; *Detwiler*, 1934.

somites over a certain range, in turn, upsets the typical separation and arrangement of the spinal ganglia in the corresponding levels; the neural crest does break up into clusters but size and distribution of these are quite irregular (Fig. 115A).

We may conclude, therefore, that the regular segmentation and seriated arrangement of the spinal ganglia is under the control of the myotomes. Why only a certain proportion of these cells stay behind to build ganglia, whereas others continue to migrate downward to form sympathetic ganglia,[1] visceral skeleton[2] and pigment,[3] is unknown.

THE HISTOGENESIS OF THE NERVOUS SYSTEM

The differentiation of nerve cells.

While the described morphological changes take place, histological differentiation has made its first strides. The cells of the medullary plate are in a condition of intermediate and transitory differentiation. Final differentiation sets in only after the cells have been released from the ependymal layer and have immigrated into the periphery of brain vesicle or neural tube. Here they begin to change in form, size, character and activity (see Fig. 13). One type, the spongioblasts, give rise to the various elements of the neuroglia. Another type, the neuroblasts, differentiate into nerve and ganglion cells of all kinds. Whereas glia cells scatter rather universally, the various types of nerve cells, particularly the more specialized ones, like the large motor cells of the cord, are found in circumscribed regions only. To inquire into the nature and operation of the factors which evoke particular differentiations in definite locations is to touch upon one of the most fundamental problems of development. But, we are as sorely ignorant of the answer in the case of nerve cells as we are in the case of other cells. We do not even know whether the cells, by the time they leave the ependyma, are still all equivalent and receive their assignments for final differentiation only later as a result of local influences to which they become subjected at the end points of their migrations, or whether they already are different when they start to move, and then direct their course toward places particularly favorable for the settlement of their kind.

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[1] *van Campenhout*, 1930.

[2] *Landacre*, 1921; *Stone*, 1929.

[3] *DuShane*, 1935; *Raven*, 1936.

The formation of nerve fibers.

All nerve cells have in common the production of one or several protoplasmic processes: *axones* and *dendrites*. It has long been a matter of dispute whether the axones are really products of the central nerve cells. Today we know that they are, but for a long time it was believed that nerve fibers arise as differentiations inside of peripheral cells and that their connection with the central cells results from secondary fusion. According to the adherents of the former view, the formation of a nerve fiber is a single continuous process comparable to the outgrowth of a seedling, while in the latter view, the fiber arises out of numerous discontinuous segments secondarily joined in tandem like the links of a chain, the whole process comparable to the laying of a pipe line. So long as the arguments in favor of one or the other contention were based merely on the microscopic study of dead and fixed objects, the issue remained unsettled. For a crucial test one had to turn to experimental methods, but even these did not at first bring a final solution, because the same facts led to diametrically opposite interpretations.

Example 110.

The theories of the central vs. peripheral origin of nerve fibers.

The experiments in question were performed quite independently by two authors.[1] Both transplanted undifferentiated limb buds of tadpoles, at a stage before nerves had appeared, into abnormal locations some distance away from the original limb site; for instance, to the head. When the transplants had fully developed, it was found that they contained nerves and that

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[1] *Harrison; Braus;* both published their first reports in 1904.

the arrangement and distribution of these nerves inside the limbs corresponded to the typical nerve pattern of a limb, no matter with what centers they were connected in the new site.

One author[1] considered this result as evidence for the peripheral origin of nerve fibers. His argument was that, if nerves were sprouts of central cells, the nerve pattern present in the transplanted limbs ought to have been that characteristic of the strange central source with which the limb was connected rather than that of a limb. An oak tree grows always as an oak, and a pine tree as a pine; therefore an eye nerve should be expected to manifest an eye nerve pattern, if it really had grown out like a tree. Since, however, the pattern was invariably that of a limb, it seemed only logical to consider the nerves as a product of the limb itself. However, this argument is not at all conclusive: the assumption that nerve fibers grow out as filamentous processes of central neuroblasts does not in any way imply that the pattern into which they grow must likewise be their own affair. The substance of the fibers might very well have come from the centers; but, when reaching the periphery it would have been poured into a mold of the making and pattern of the limb. This was, indeed, the conclusion at which the other author[2] arrived. At any rate, these experiments did not yet bring the expected clarification. It seemed impossible to obtain definitive information about the respective parts played by centers and periphery as long as they could not be rigorously separated from each other. *Harrison*, then, made the crucial step and separated them.

Example 111.[3]

Free outgrowth of nerve fibers in tissue culture.

A fragment of spinal cord of a frog tadpole was excised and replaced by a blood clot. When this clot was later studied it was found to be pervaded by nerve fibers. Since these could not have originated in the dead substance of the clot, it was clear that they must have *grown into the clot as extensions of central nerve cells* of the vicinity. Finally, to insure complete control of the conditions, fragments of spinal cord were removed altogether from the body and embedded in cell-free clots of lymph in vitro. Not only did the explanted fragments survive, but their vitality remained undiminished for days; thus, the method of tissue culture was created (see p. 153). From the cells of the explant, however, *fine protoplasmic processes were seen to grow forth*, giving rise to veritable axones and dendrites and revealing to the observer not only the fact of their outgrowth but, at the same time, its mode and rate.

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[1] *Braus*, 1905.

[2] *Harrison*, 1907a.

[3] *Harrison*, 1907b, 1910a, b.

By these classical experiments it has been definitely established that the conducting part of a nerve fiber, and of every nerve fiber, is in its entire length a protoplasmic sprout extending from a single neuroblast cell. Subsequent direct microscopic observations on nerve development in the living organism (amphibian larvae), facilitated by the transparency of the young larval tissues, have amply confirmed the results of tissue culture (Fig. 116).[1] There is no other mode of nerve formation but by outgrowth.[2]

The origin of sheath cells.

The process sprouting out from the nerve cell corresponds to the later axon or dendrite of the adult nerve fiber. It develops neurofibrils in its interior and contains the essential mechanisms of excitability and conduction. Throughout its peripheral course it is surrounded by a sheath of foreign protoplasm which belongs to very elongate cells applied to the fiber in tandem alignment (sheath cells of *Schwann*). It was chiefly the ubiquitous association of these cells with the nerve fibers that had misled so many observers to the conception of a peripheral origin of the nerve fiber, with

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[1] *Speidel*, 1933.

[2] *Braus*, yielding to the overwhelming evidence in favor of the outgrowth theory, later (1911) accepted it and reinterpreted his earlier experiments accordingly.

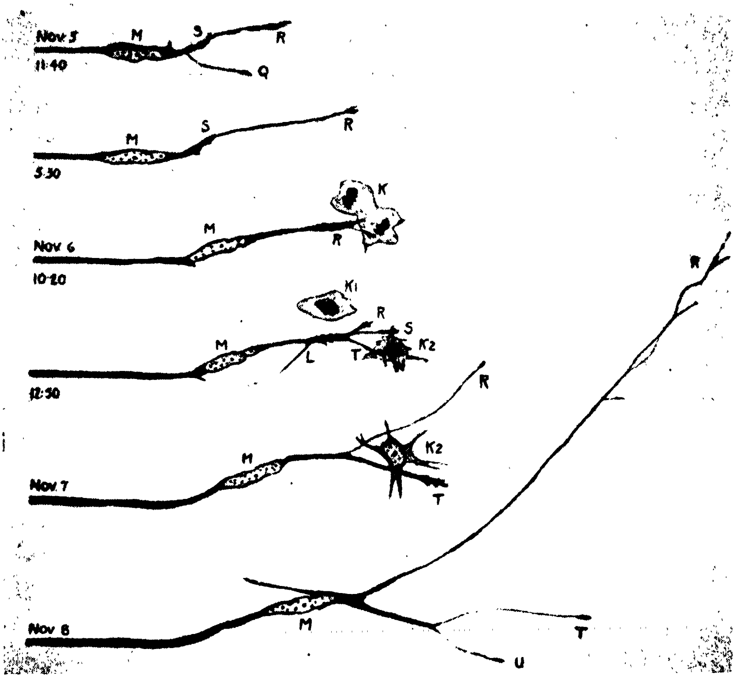

Fig. 116. Advance of the distal end of a growing nerve fiber with associated sheath cell (M) in the regenerating tail fin of *Triturus viridescens* over a period of three days. (From *Speidel*, 1935)

R, Most actively growing sprout. Q, L, S, Unsuccessful sprouts presently withdrawn. K, Mitotically dividing connective tissue cell. The mitotic act has apparently irritated the near-by nerve tip and caused it to branch into R, S, and T.

the sheath cells as the supposed source. Today we know that these cells, although of central origin themselves, cannot produce nerve fibers; they merely attach themselves secondarily to the nerve fibers like ivy to the trunk of a tree. This fact has been demonstrated by the following experiment.

Example 112.[1]

In a frog embryo the dorsal portion of the early neural tube was excised along with the neural crest. Spinal ganglia and

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[1] *Harrison*, 1924; *Detwiler*, 1937.

> sensory nerves failed to develop, because their source, the ganglionic crest, was missing; but motor nerves grew out from the ventral portion of the cord as usual. *These motor fibers were naked*, however, lacking the customary coat of sheath cells.

These results, in addition to proving once more the essential irrelevance of sheath cells for nerve fiber formation, have led to the conclusion that sheath cells are predominantly products of the neural crest. Later work has suggested that the source may not be so strictly circumscribed and that cells originating in the ventral portion of the spinal cord may also emigrate to become sheath cells, particularly during later stages.[1] At any event, sheath cells are of central origin, but devoid of neuroblastic faculties.

The intimate association between axon and sheath cell must be due to some specific affinity between their protoplasms, the nature of which is still wholly obscure; for convenience, one may call the relation a "tropism," with the understanding that this merely states the fact without elucidating it in the least. The cylindrical myelin sheath, developing in certain types of nerve fibers at the boundary between the axon and the enveloping sheath cells seems to be produced by the former, probably in response to a stimulus by the latter.[2] Within the central nervous system, where sheath cells are lacking, myelinization is achieved with the aid of the neuroglia cells.

The individual nerve fibers, each consisting of axon and enveloping sheath of *Schwann*, are then secondarily assembled and cemented together into bundles, constituting the nerves. The cementing material is fibrous connective tissue, mesodermal in origin.

THE FORMATION OF NERVE PATTERNS

The basic features of nerve patterns.

If we disregard, for the time being, the accessory structures, such as sheath cells and connective tissue, and focus on the axon as the main constituent of the fiber, we note the following outstanding features:

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[1] *Raven*, 1936; *Jones*, 1937. [2] *Speidel*, 1932.

1. The number and kind of processes produced by a given neuroblast are characteristic for that particular type of cell. The first process to emerge generally becomes the axon. All later processes turn into dendrites (with such exceptions as the originally bipolar cells of the spinal ganglia).

2. Certain groups of axons leave the central nervous system and penetrate into the peripheral tissues, while others travel intracentrally for varying distances. The course of some processes is oriented in definite directions, while others seem to stray at random. The direction in which a fiber proceeds depends upon the type of fiber, the location and the developmental stage. The complicated design in which the central and peripheral nerve fibers are observed in the developed organism reflects the variety of courses taken by the fibers at the time of their outgrowth.

3. Peripheral fibers are assembled into bundles in numbers fairly typical for the particular nerve. In definite places neighboring nerve trunks may anastomose to exchange fibers (formation of peripheral plexus); in definite places the larger bundles branch into smaller ones continuing along separate routes; and, finally, the fibers terminate and establish functional connections with definite non-nervous tissues. Thus is established what we call the typical *nerve pattern.* Though it varies slightly among different individuals, the difference is decidedly less than the difference between the branching patterns of any two specimens of trees of the same kind and growth.

4. The peripheral connections are effected in such a manner that the various receptor and effector organs (sensory cells, muscle fibers, glands, etc.) are, on the whole, correctly united with corresponding parts in the central nervous system. How much latitude there is, we do not know, but we do know that nerve fibers coming from spinal ganglia are connected with sensory end-organs, fibers from

the motor horns of the spinal cord with cross-striated muscles, sympathetic fibers with smooth muscles and glands; in short, each fiber type with its appropriate type of end-organ.

Mindful of the generally composite nature of developmental processes as we have come to know them, we are not surprised to learn that these several features are brought about by just so many, or even more, different factors. We have begun to realize that it is unwarranted and devious to search for a single agent as *the* supposed "determiner" of a nerve pattern.

Singularity or plurality of pattern-determining factors?

Formerly, authors speculating upon the factors controlling nerve development were rather exclusive in the choice of agents they believed to be involved. Some assumed them to be electrical in nature,[1] others considered them to be a chemical [2] or metabolic affair,[3] still others favored a mechanical interpretation;[4] and many of these hypotheses claimed general applicability to the exclusion of all others. The migration of the neuroblast, the establishment of its polarity, the production of its processes, the course of the outgrowing fibers and, finally, the conditions of their termination, all these were thought to be under the control of one single kind of agent, represented by either differences in electrical potential or diffusible chemical substances or gradients of metabolic activity or mechanical influences. There is nothing, however, in the facts to warrant such limitation of viewpoint. When the phenomena listed above — to which many more could be added, if we were to treat the subject more comprehensively — are subjected to experimental analysis, one soon discovers that there is not one, but a variety of factors at work. This we intend to demonstrate in the following.

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[1] *Strasser*, 1892; *Kappers*, 1917.

[2] *Cajal*, 1893.

[3] *Child*, 1921.

[4] *His*, 1887; *Harrison*, 1910a.

The polarity of the nerve cell.

That the production of a protoplasmic process is an intrinsic capacity of the neuroblast has been proved by the tissue culture experiments referred to above: isolated neuroblasts, completely released from the influences of the organism and severed from all relationships with their normal surroundings, still develop nerve fibers spontaneously. In other words, the tendency to produce axonic and dendritic outgrowths must have arisen in the cell during an earlier stage of its career, evidently as part of its assignment to neuroblastic differentiation. Yet this does not imply that the exact locations on the cell surface where the processes are to emerge are likewise predetermined by properties of the cell; it is conceivable that conditions extrinsic to the cell might decide about this point.

There is a theory contending that the neuroblast becomes "polarized" by an electrical gradient; the part of the cell facing the anode forms a protrusion: the axon.[1] One could imagine that a local electrical change on the surface of the neuroblast might create a "weak spot" through which protoplasm could bulge out and establish an axonic outgrowth. Although this contention may be correct, no direct proof for it has been forthcoming. It must be kept in mind, however, that the factor determining the place at which the axon leaves the cell body, whether electrical or not, does not at the same time make arrangements for the orientation and the further progress of the emerging fiber. Once the fiber has burst forth from the cell its further guidance becomes a matter of certain influences which, as we shall see, are certainly not merely electrical in nature.

The mode of outgrowth of the nerve fiber.

The nerve process, after leaving the cell body, continues to grow, threading its way through the new environment. The elongation of the process does not seem to be brought about by the squirting out of more axonic substance from the cell body, as one squeezes cream from a tube, but is due apparently to the pull exerted by the actively advancing tip upon the rest of the fiber. This tip, endowed with amoeboid motility, proceeds into the medium, spinning out the fiber behind it and siphoning more and more substance from the cell body. While the rate of growth varies considerably according to circumstances, its maximum may be estimated to be of the order of 1 mm. per day.

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[1] *Kappers*, 1917.

This mode of growth makes it plain that a neuroblast, though being the source of the axon, cannot also provide its itinerary. A growing fiber has no inherent tendency to move in any particular direction, nor to change its direction at certain points, nor to branch and terminate after having reached a certain length. For all these actions it is dependent on directive influences of the environment, and its own endowment in this respect consists merely of the faculty of letting itself be guided. Under conditions in which directive extraneous influences are absent or unorganized, as for instance in an ordinary tissue culture, the axonic growth runs wild, ending up in a chaotic entanglement of fibers instead of in a regular pattern (see Fig. 118A). Evidently, a fiber tip after having emerged from the cell body needs a guide for further directions.

What factors, then, serve in this capacity? Does the growing tip perhaps somehow perceive the distant place of its destination and strive to get there in any practicable way? Or does it follow whatever route it finds open, drifting aimlessly to a chance termination? Both viewpoints have been advocated.

The chemotropic theory of nerve orientation.

Some authors have contended that *specific chemical emanations*, produced by the various peripheral tissues and diffusing toward the centers, serve to attract the outgrowing fibers, just as the scent of a flower attracts an insect.[1] One used to refer to the classical experiments of *Pfeffer*, supposedly analogous, in which spermatozoa of ferns suspended in water were seen to swim toward a capillary tube containing malic acid, while sperm of moss could be attracted by diffusing sugar. Phenomena of this kind have been called "*chemotaxis*" or "*chemotropism.*" "Chemotropic" attraction of a nerve fiber by some distant source of diffusion would depend upon many prerequisites. First, on the part of the peripheral tissues: production of a great variety of different substances so as to enable different fiber groups to take specifically different courses; mutual noninterference of these substances; undisturbed diffusion, as in a stagnant pool, because if the medium were constantly stirred, the concentration gradients which are to indicate the way toward the center of diffusion would be so thoroughly distorted that they would become will-o'-the-wisps rather than guides to the confused nerve fibers. Second, on the part of the fibers: a delicate chemical sense allowing the growing tip to discriminate the one attractive substance intended for it from amongst the enormous variety of chemical products present in the tissue spaces; furthermore, means to recognize precisely the direction in which the source of the emanation lies. If only one of these prerequisites failed to materialize, chemotropic orientation could not take place. As a matter of fact, the conditions under which the outgrowth of a nerve fiber occurs fail to meet several of these postulates; which makes untenable the theory, that "neurotropism" (directed nerve growth) is a form of "chemotropism."

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[1] *Cajal*, 1908; *Forssman*, 1898, 1900.

The electrical theory of nerve orientation.

Another group of authors favored an *electrical* theory;[1] the same potential differences that were claimed to be responsible for the polar orientation of the neuroblast and which, as we have said before, may very well be instrumental in fixing the place at which the fiber emerges, were also assumed to control the direction of its further advance.

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[1] *Strasser*, 1892; *Kappers*, 1917; *Ingvar*, 1920.

Fibers would travel in a one-way sense along the lines of an electric field generated by the embryo. This theory of "galvanotropism" not only leaves many facts unexplained, but is plainly contradicted by a number of observations and experiments.

Counter-evidence obtained from direct observation is illustrated by the following.

Example 113.

When the progress of growing nerve fibers in the tail of immobilized living tadpoles is watched under the microscope, one sees occasionally two axones moving along the same path but *in opposite directions.*[1] The same has been known to occur regularly in the development of the brain where sometimes reciprocal fiber tracts between two centers arise simultaneously.

It is obvious, that if nerve fibers were directed toward regions of definite electrical sign, there could never be simultaneous growth in opposite directions; since such antagonistic growth has, however, been observed, electric gradients cannot very well have been the directive agents.

Counter-evidence from experiments:

Example 114.

Fragments of embryonic central nervous system in tissue culture displaying abundant outgrowth of nerve fibers were exposed to the electric current by a great variety of methods. In no case, where the experiments were done critically and with the necessary precautions, have the fibers shown any trace of orientation with regard to the electric current. If growing at all, they followed *random courses,* quite unaffected by the presence of the electric field.[2]

Consequently, the foundation of the *galvanotropic* theory is not any more solid than that of the theory of chemotropism.

[1] *Speidel,* 1933.

[2] *Weiss,* 1934c; *Karssen and Sager,* 1934. Negative results have also been reported by *G. Levi,* 1934, p. 611, and *S. C. Williams,* 1936. The effects mentioned by *Ingvar,* 1920, were presumably of other than electrical origin (cf. *Weiss,* 1934c; p. 426).

"Attraction" of nerves by developing organs.

On the other hand, in spite of all these negative results, there is the incontrovertible fact that nerve fibers do not just happen upon their terminal fields but are somehow directed toward them through what appears to be an "attractive" influence.

Example 115.[1]

In an amphibian embryo of a stage prior to the outgrowth of nerves, a fore limb bud is transplanted to a position a certain distance away from its original site. The distance may conveniently be measured by the number of somitic segments it includes. The normal extent of a limb bud is from the anterior border of the third to the posterior border of the fifth segment. From the experiments reported in example 110 we know that a transplanted limb bud will obtain full innervation. But where do its nerves come from? Are they formed by the fibers originating at the level of the spinal cord corresponding to the new site of the limb, or are they composed of fibers from the original limb segments? In the latter case the fibers, in order to reach the shifted limb, would have had to deviate from their normal course. The experiments have shown that neither of these views is wholly correct; the truth lies somewhere in the middle. A limb shifted to a more anterior or more posterior position obtains its nerve supply from a farther anterior or posterior level of the spinal cord, but the shift of the nerve source is never as great as that of the limb has been. If, for instance, a limb has been moved caudad for three segments, its nerves may come from a spinal level situated only one or two segments posterior to the normal limb segments. This means that the nerves must have been slightly *deflected from their ordinary course*, anteriorly if the limb was shifted headward, and posteriorly if it was transplanted caudad.

Thus one obtains the impression that a growing limb bud exerts an "attracting" influence on nerves. The fact that this influence is rather general and *unspecific* has been brought out quite clearly by a number of observations. *Limb* or *trunk* nerves are found to converge upon a *nasal* placode transplanted to the flank [1] (Fig. 117); they are similarly "attracted" by a growing *tail* bud or, less markedly, by a developing *eye*.[2]

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[1] Summarized in: *Detwiler*, 1936.

Growth rate and "attractive" power.

Evidently any *localized area of intensive growth* has the ability to "attract" nerves which happen to develop during its burst of activity. In this sense it has been very revealing to find that nerves allowed to grow toward the brain instead of toward the periphery behave in the same manner. If a nasal placode whose sensory cells develop nerve fibers is transplanted to another head region, its fibers grow into that part of the brain where at the time mitotic activity is at a peak.[3] So, the fibers do not select a course toward specifically related parts, peripheral or central, but grow toward any region in which there is intensive proliferation, regardless of whether the connection thus established is

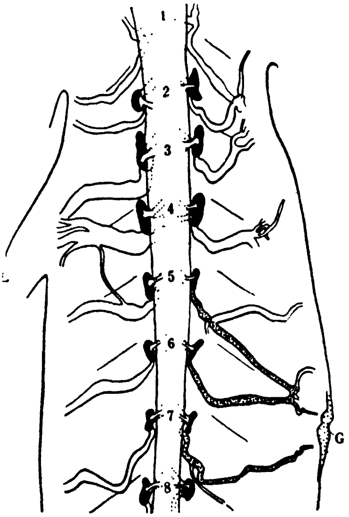

Fig. 117. Convergence of nerves upon growing organs. (After *Detwiler*) Nerves 5, 6, 7 (stippled) converge upon grafted nasal placode (G). The right limb bud had been extirpated. Note the smaller size of the nerves of the limb plexus (3, 4, 5) on the limbless, as compared with the normal left side. L, left limb. × 14.

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[1] *Detwiler*, 1928.

[2] *Detwiler and Van Dyke*, 1934.

[3] *Burr*, 1932.

right or wrong. Quite in line with this behavior of peripheral nerves, one finds that the growth of tracts of nerve fibers within the brain is also directed toward points of increased developmental activity,[1] and if locally intensified activity is present at the same time in two or more centers, these become subsequently interconnected by fiber tracts. In this way the mitotic pattern of the brain lays down some essential traits of the intracentral fiber pattern (p. 507).

The stereotropic theory of nerve orientation.

Summing up our evidence, we must admit that there are definite directive, even "attractive," forces at work, but they are neither chemotropic nor galvanotropic. What else are they? There is still the "*mechanical*" viewpoint left to be considered.

A long time ago, attention was called to the marked tendency of embryonic nerves to move along solid structures such as fibers, blood vessels, or skeletal surfaces. Some authors picture nerve fibers as proceeding along fine protoplasmic filaments (plasmodesms) connecting the cell layers of the embryonic body.[2] The protoplasmic nature of these filaments is disputed, but this is rather immaterial in the present connection. The point of interest here is that nerve fibers seem to apply themselves to mechanical structures of their surroundings and to follow them. A similar behavior has been known to be exhibited by certain migratory cells and has been designated as "thigmotaxis" or "stereotropism." If the growing tips of nerve fibers should prove to be as apt to be guided by contact as are, for instance, mesenchyme cells (p. 451), their course would be a mere matter of the configuration of their immediate surroundings. Nonnervous structures would serve as rails along which the nerve tips would travel, and nerve patterns would in last analysis resolve themselves into pre-neural patterns, retraced and thus duplicated by nerve fibers. This conception, if correct, reduces the problem of nerve orientation almost completely to a matter of the oriented guiding substratum.

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[1] *Coghill*, 1929.

[2] *Held*, 1929.

Ultrastructures as guides of nerve fibers.

It might be objected that the contact between nerve fibers and non-nervous structures of *macroscopic* or *microscopic* visibility, while a frequent occurrence, is by no means the rule. This is true, but the difficulty disappears if we include *submicroscopical* structures in our consideration. Then, we may envision the orientation of the nerve fiber as being achieved by a principle essentially similar to the one described above as orienting the growth of mesenchyme: *ultrastructural organization* (p. 453).[1] Again, basic information has come from tissue culture experiments.

Example 116.[2]

> As mentioned before, nerve fibers when growing out under the standard conditions of tissue culture, i.e., in a clot of blood plasma not otherwise treated, swarm out along irregular, crooked, haphazard courses (Fig. 118A). An entirely different picture is obtained if the coagulating culture medium has been stretched or stroked with a brush in a constant direction. In that case the *nerve fibers grow out strictly parallel* (Fig. 118B), oriented in the direction in which the clot had been stretched or stroked.

The effect of the mechanical treatment upon the coagulating medium is known: the ultramicrons are forced into an orientation more or less parallel to the acting force; as a result, they aggregate into threadlike units (see p. 33). Thus, a longitudinal *ultrastructure* is created in the medium which in fixed and stained preparations can be recognized as a faint striation. It is by following this ultrastructure that nerve growth has become oriented. The fibers, striking upon the ultrastructural pathways in the medium, move along them, like trains on parallel tracks (Fig. 99).

Of the reason why growing tips of nerve fibers are bound

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[1] *Weiss*, 1933.

[2] *Weiss*, 1934c.

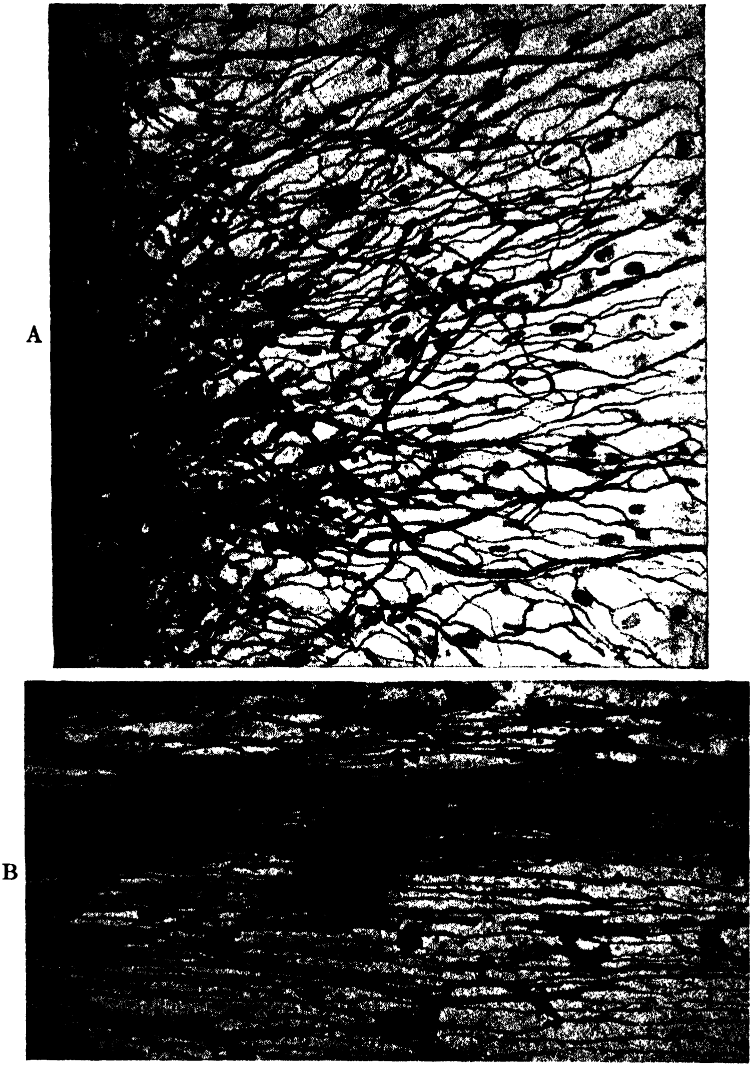

Fig. 118. Effect of the ultrastructure of the medium on the orientation of nerve growth in vitro.

A, Growth zone of an ordinary mixed culture of nerve cells and mesenchyme (spinal ganglion of chick embryo) showing the random course of the nerve fibers.

B, Strictly oriented growth of nerve fibers in a clot which had been subjected to stretch (in the horizontal direction). × 280.

Direction of growth in both pictures from left to right.

to travel along submicroscopic or microscopic surfaces or interfaces, we are wholly ignorant. All we know is that they do it, and that, wherever in their growth they find a properly oriented substratum, they will translate its orientation into their own. We may call their behavior "thigmotaxis"; this is a good term, but it explains nothing, and further analysis of the matter is badly needed. In the meantime, one may assume that the surface film of the protoplasm of the nerve tip possesses specific properties which make it spread out along the interfaces between the fibrillar solid and the capillary liquid of the colloidal medium; a vague analogy is the spreading of oil on the phase boundary between air and water. If thigmotactic affinity should turn out to be a specific character differing for various kinds of protoplasm, this would admit of a certain degree of electivity, inasmuch as one type of protoplasm would possess adhesiveness to a given structure and follow it, while another type would not; just as a dry thread sticks to adhesive tape while a wet thread does not. This could explain why nerve fibers do not all follow the same structures.

If we agree to call this guidance by contact of the nerve fiber a "mechanical" effect, then the "mechanical" factor seems to be the only one to have an immediate influence on the course of the nerve fiber. All other agents must act through its mediation, that is, they can become effective only to the extent to which they influence the pathway structure. On this assumption many peculiarities of developing nerve patterns have found an easy and adequate explanation.

The pioneering stage of fiber outgrowth.

Before we describe some pertinent examples, attention must be called to two facts which are sometimes overlooked. The first is that at the time when the primordial nerve patterns, both peripheral and intracentral, are first established in the embryo, the nervous elements constituting them are *very few in number;* and the second is, that the *distances* which these first fibers must span before arriving at their destinations are *very short.* Only during the later stages of embryogenesis and in postembryonic life, do the elements reach the comparatively enormous numbers and dimensions characteristic of the functional animal. It is important to keep this in mind because the factors establishing the elementary pattern are not identical with the ones elaborating it later to its final perfection. Only the early pioneering fibers are confronted with the task of finding their way into the unexplored country of peripheral tissues; for this reason they have been called "*pathfinders.*" Most fibers produced at a later time follow the courses chosen by the earlier ones; apparently fellow nerve fibers assume a similar guiding role as was originally played by non-nervous structures.

The passive elongation of fibers.

At the time when the pathfinders swarm out, the embryo is still small, its various organs lie close together, and a fiber does not have to travel very far to reach the periphery. For instance, the first motor fibers of the spinal cord grow out into the myotomes at a time when the latter and the neural tube are still in close apposition. Similarly, the first limb nerves reach the limb when it is still an inconspicuous bud. After spanning these comparatively short distances the tip of the fiber becomes attached to a peripheral tissue cell, and this puts an end to its roaming. Henceforth, instead of advancing freely, the fiber is taken in tow by the tissue to which it has become joined. This has important consequences for the fibers; for the peripheral tissues undergo considerable displacements owing partly to unequal growth, partly to active movements which they perform relative to each other. The muscle buds, for instance, migrate over considerable stretches, trailing their nerve fibers behind. Such a fiber, with one end anchored in the central cell and the other end hitched to a peripheral organ, is no longer free to choose its way; its later course indicates the route traveled by the structures with which it has become connected, rather than the direction of its own original outgrowth.

Three phases may, consequently, be distinguished in nerve development: (1) the phase during which the pioneering fibers proceed actively and take a free course; (2) the phase during which they are taken in tow by some peripheral organ and passively dragged along; (3) the phase during which post-pioneering fibers grow out, advancing along the the surface of the former pioneers which have become settled by this time. It is obvious that the problem of how a nerve fiber finds its way exists only for the first and third phases, during which the fibers are moving actively. Since during the third phase fibers merely duplicate courses established by earlier fibers, the fundamental problem of nerve orientation narrows down to a consideration of what happens during the first phase. At that time the embryo is still in a comparatively early stage of development, and the distances to be spanned by the young fibers are infinitely smaller than they appear in later life. It is in this setting that the *ultrastructural organization* of the embryo assumes prime importance in determining the basic nerve patterns, of which we shall now present a number of specific examples.

The origin of ultrastructures.

In order to understand our further discussion, it is important to remember the following points: (1) Oriented mechanical tension applied to a colloid, particularly one containing rod-shaped elements, forces the latter into oriented arrangement (ultrastructure; detectable by polarized light). (2) If the orienting agent endures without changing its direction, the ultrastructure will gradually become consolidated and may reach microscopic dimensions (microscopic structure; detectable by histological stains). (3) If the orienting agent changes its direction, (a) before an advanced stage of consolidation has been reached, the existing labile ultrastructure may be destroyed and superseded by a new one corresponding to the changed orientation; (b) after consolidation has surpassed a certain limit, the old structure will persist. (4) Tensions arise within the ground-substances of the body chiefly as a result of (a) growth in general and particularly unequal growth of different parts; (b) movements of some parts relative to others; (c) local changes in size owing to swelling or shrinkage. (5) Both swelling and shrinkage are the results of certain physical or chemical changes altering the properties of the colloids. Consequently, all physical or chemical agents conducive to such changes are on the same score potential molders of ultrastructural patterns and, in further consequence, of nerve patterns.

Ultrastructures as explanation of the "attraction" of nerves.

As examples of how these facts apply to the mechanism of nerve pattern formation, two striking phenomena may be selected: (1) the *attraction of nerve fibers* toward certain areas of the embryo, as described above, and (2) the branching of fibers and the *formation of nerve plexus.*

We have said (p. 525) that nerve fibers tend to converge upon active centers of proliferation, as if "attracted" by them from over a distance. Yet, if we accept the view that nerve fibers are solely directed by the contact with their immediate surroundings, the possibility of direct "attraction" from a distant source must be flatly denied. Consequently, some way must be found to explain the apparent "attraction" effect in terms of ultrastructural organization. This is relatively easy; all one would have to demonstrate is that foci of proliferation cause the ground-substance within their province to assume a structure oriented toward them; the nerves would automatically follow in the same direction. Tissue culture experiments have shown that this is precisely the situation into which the phenomenon of "attraction" resolves itself.

Example 117.[1]

Reciprocal nerve growth in tissue culture.

Two embryonic spinal ganglia (chick embryo) were explanted at considerable distance from each other (several millimeters) into a membrane, cca. 0.05 mm. thick, of coagulated blood plasma. Each ganglion, composed of nerve cells, sheath cells, and mesenchyme cells, becomes the seat of twofold activities. The nerve cells *differentiate* and give off axons which penetrate beyond the original limits of the explanted fragment into the surrounding medium; while the accessory, non-nervous, cells, in addition to migrating out, *multiply* very actively by mitotic divisions. Under these conditions, outgrowth of the nerve fibers occurs (Fig. 119) in spectacularly *straight orientation along the line connecting both cultures*. Fiber growth is reciprocal, i.e., fibers from either ganglion advance toward the other.

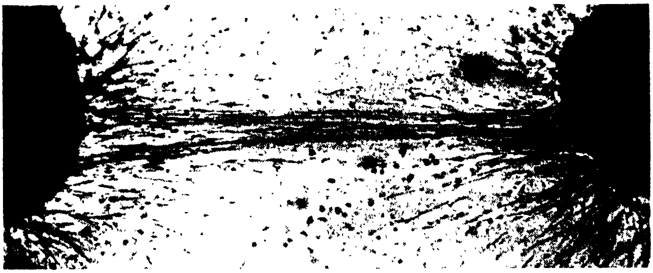

FIG. 119. Oriented nerve growth connecting two centers in vitro. (From *Weiss*, 1934) Two spinal ganglia of a chick embryo (dark bodies in the picture) were explanted in a blood plasma film at a distance of cca. 4 mm. A straight strand of spindle cells and nerve fibers formed along the connecting line within 3 days of cultivation. × 45.

On the surface this looks as if the ganglia had mutually attracted each other's fibers. In reality, however, the fibers have by no means "sensed" the presence of a ganglion at the far end of their course, but have been passively guided in that direction by a peculiar structure established in the plasma clot by the simultaneous growth of the two centers. The actual sequence of events as revealed by experimental analysis is this: (1) The proliferation of non-nervous cells contained in the explants discharges dehydrating substances into the surrounding medium. (2) These cause local dehydration of the plasma clot in the vicinity of the two ganglia. (3) The dehydration (loss of liquid) causes shrinkage (comparable to gradual drying out of a jelly by evaporation). (4) The local shrinkage around the two centers sets up tensions, the major directions of which can easily be ascertained by the use of suitable models: the resultant tension is oriented along the connecting line of the centers. (5) The oriented tension forces the plasma coagulum to assume a correspondingly oriented structure; thus a bridge is formed consisting of more densely aggregated parallel ultramicrons connecting both centers in a straight line. (6) The nerve fibers growing out from either end find the stage all set; they simply proceed along the bridge and are thus conducted toward the opposite center.

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[1] *P. Weiss*, 1934c.

The role of ultrastructural organization in producing results which simulate "attraction" effects has thus been definitely proved. It may be mentioned that certain aspects of these experiments have, at the same time, furnished incontrovertible evidence that no form of direct attraction over a distance has had a part in the result.

Ultrastructural nerve orientation in the embryo.

Viewed in the light of these model experiments, related situations in the embryo can be satisfactorily explained. We now readily understand why nerve fibers are always directed toward *growing* organs and why, on the other hand, this apparent "attraction" is *unspecific,* i.e., determined by the growth rate rather than by the character of the organ in question (see p. 524). Wherever a localized area begins to grow at an increased rate it will inevitably set up tensions in the surrounding portion of the embryo, either directly or owing to the dehydrating effect of proliferation (4). These tensions must, on the whole, converge radially upon the active center, and the ground-substance will be forced into a corresponding orientation. Thus pioneering fibers, when happening upon this ground, will of necessity be turned into the prepared radial course and be conducted toward the growing center, be this a limb or a tail or a nose.

These experiments also furnish an explanation of the fact that those centers of the early brain which develop simultaneously become interconnected by nerve fiber tracts (see above p. 507). To understand this, one has simply to visualize the ganglionic explants of our experiments as representing two such brain centers of increased growth activity, and the plasma medium as representing the ground-substance of the brain. Other things being equal, the brain matrix and hence fiber growth must necessarily become oriented along the connecting line between the active centers. It is important, however, to remember that the distribution of growth centers is not as stable in the embryo as it is in the tissue culture experiments. We have reported above (p. 506) that the growth pattern of the embryonic brain changes continually. Each new constellation of growing foci yields a new system of tensions and a corresponding ultrastructural pattern. Each transitory configuration of the ground-substance, however, leaves a permanent residue in those nerve fibers which have grown out during its term. So, as the embryo grows older, one nerve pattern after another is woven into the texture of the brain, and an ever increasing intricacy of lines is produced. The developed brain finally could be compared fittingly to a page on which numerous texts have been printed one on top of the other — and is just as difficult to decipher. This consideration gives concrete meaning to the statements we made above on page 491.

The branching of nerve fibers.

Outgrowing nerve fibers do not generally remain simple. Sooner or later they branch so that, eventually, each axon and dendrite is split into numerous terminal twigs up to several hundred. Branching occurs in two ways. *Mode a:* the growing *tip* of a fiber splits into two sprouts both of which continue to move as separate processes to become later subdivided again and again; the result is a more or less extensive arborization. *Mode b:* lateral excrescences — so-called collaterals — bud out from the *stem* of an established nerve fiber. Observations on living fibers suggest that collaterals may be produced anywhere along the stem of a fiber in response to a local irritant; at the weakened spot a lateral bulge of protoplasm appears which may give rise to a side branch provided with a new growing tip.

Mode *b* reminds us of the production of a new hydranth from the body wall of a hydroid after local injury (see example 72, Fig. 79). Branching according to mode *a* seems to follow a different method. When an advancing growth cone strikes upon an oriented structure lying across its way it will be deflected into this crossroad. If the crossroad extends in both directions, some of the pseudopodia of the growing tip will turn to the right and others to the left, and as a result the fiber will be pulled apart. This is quite frequently observed when an advancing fiber encounters an accidental obstruction, e.g., a cell or another fiber (Fig. 116). In order to explain the fact that in normal embryology the branching of fibers occurs regularly in typical places rather than at random, one would have to assume that the typical order of embryonic events has provided for the appearance of obstructions in definite locations to bar the straight advance of the fibers.

Let us consider the following example: the sensory process of a sensory nerve fiber, the cell body of which lies in the spinal ganglion, grows more or less perpendicularly into the dorsal portion of the spinal cord; there it splits into two branches which take a lengthwise course (Fig. 120), one ascending and the other descending the cord. Although there is no direct proof for it, the assumption is obvious that both the bifurcation and the deflection of these fibers is the result of one and the same condition, namely, the fact that at their entrance into the cord they strike upon the longitudinal structure of the dorsal columns of the spinal cord. A jet of water directed at right angles against a gutter illustrates the essential point.

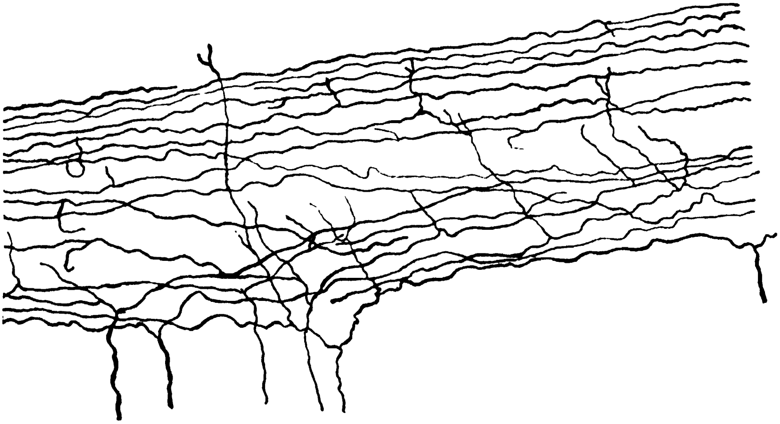

FIG. 120. Course of dorsal root fibers upon entering the spinal cord (chick embryo). (After *Cajal* from *Lillie*)

Six fibers coming from the spinal ganglion (below) are shown as they enter the dorsal longitudinal tract and, after branching, are deflected into the general longitudinal course.

Ultrastructural basis of plexus formation.

If we accept this suggestion, branching may be considered as a peculiar by-product of the deflection of fibers obtaining under certain conditions. The same seems to hold for *plexus formation.* Fibers coming from all directions when forced into a common orientation by a strip of strictly oriented matrix crossing their ways must necessarily associate with each other. Thus cross structures enable nerve fibers from different sources arriving from different directions to intertwine and mingle (plexus in the various strata of the brain) or to recombine in new groupings (girdle plexus of the limbs). Cross structures can arise in various ways; one simple example may be mentioned here.

Example 118.[1]

In tissue culture two clots of blood plasma of markedly different dilutions are joined together. A nerve culture is embedded in one of them. Its fibers grow out. As long as they are in homogeneous medium they grow in all directions until some of them arrive at the boundary zone between the two clots. There they are noticeably deflected from their original courses into an orientation parallel to the border zone. *A rather dense plexus along the border results* (Fig. 121).

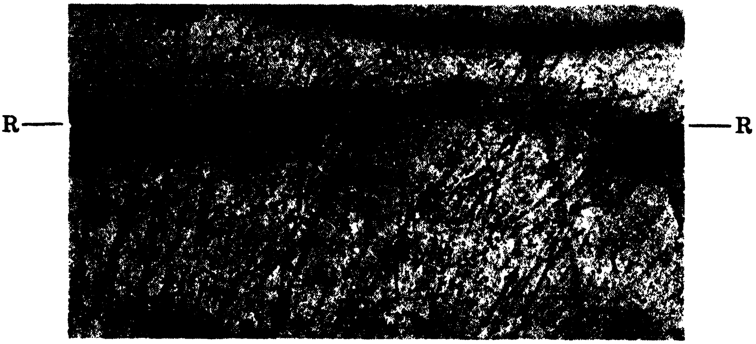

FIG. 121. Deflection of nerve fibers. (From *Weiss*, 1934)
Photograph of nerve fiber plexus forming at the boundary of two plasma clots. The fibers have first grown out in the general radial direction of the arrows. Coming upon the border zone (R–R, recognizable by its darker stain), they have turned into the tangential course of the boundary. (Compare with Fig. 120.)

The explanation is as follows: the plane along which the two clots are fused forms a sort of partition, and the ultramicrons of the medium settle, for reasons which we cannot detail here, in an ultrastructure parallel to that plane. Accordingly, nerve fibers arriving there are deflected from their predominantly centrifugal courses into the tangential orientation of the border zone. This result seems of particular interest because it might explain the origin of the various plexiform layers in the brain. On an earlier occasion (p. 509) we have mentioned the possible cause of the fundamental lamination of the young brain; we have indicated that the various strata differ in their colloidal character. If this is correct, then any two adjoining brain strata are in the same situation as the two fused plasma clots of our last experiment. The strata will assume at their mutual boundary ultrastructural organization oriented parallel to the plane of separation. Consequently, radial fibers arriving at the surface of a stratum will be turned into this plane and form a plexus.

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[1] *P. Weiss*, 1934c.

The formation of the brachial and lumbo-sacral plexus of the limb nerves of vertebrates are perhaps traceable to a similar origin. The orientation of the ground substance in which the pectoral and pelvic girdles arise is essentially parallel to the body wall, which means that it lies across the main nervous pathways. Under these conditions, it is not surprising to find the nerve fibers intermingling at this level and thus giving rise to the anastomoses of the plexus, while after having overcome the obstruction they continue in a peripheral direction.

We have endeavored to show in these few examples how *ultrastructural organization* can affect nerve growth, and does affect it *in vitro*. There is a host of observations indicating that the principle is essentially the same *in the embryo*. It is left for future work, however, to determine the limits of its applicability.

The terminal connections of nerve fibers.

Of the three sections into which the journey of a nerve fiber can roughly be subdivided — (1) projection from the central nerve cell; (2) peripheral course; and (3) terminal connection with peripheral cells — the first two have been considered in the preceding pages. It remains to say a few words about the third.

It was pointed out above (p. 492) that in the developed body every peripheral tissue is innervated by an appropriate set of nerve fibers; for instance, sense organs by afferent fibers whose cell bodies lie in the spinal ganglia, and cross-striated muscles by efferent fibers issuing from the ventral portion of the spinal cord. Furthermore, the particular manner in which a nerve fiber is connected with its end-organ is specific for the type of tissue innervated; the histological structure of the junction between a motor nerve fiber and a muscle fiber is markedly different from that of the terminal connection between a sensory fiber and its sense organ. In other words, the functional differences distinguishing peripheral organs are, at least partly, reflected in gross differences of the terminal apparatus of their innervating nerve fibers. This fact, well established for the developed nervous system, must of course rest upon an embryological foundation, and our interest turns to the question of what factors might possibly be responsible for bringing about such selective connections between nerve fibers and end-organs as the finished organism has been shown to possess. Up to the present, no satisfactory solution of the problem has been found; largely perhaps, because no adequate efforts have as yet been made to solve it. Several possibilities suggest themselves almost as a matter of course, but most of them have proved to be not consistent with the facts. Let us briefly survey them.

Does selectivity prevail in the establishment of nerve connections?

1. Selective connections might be assumed to be a result of selective attraction; each type of tissue would have to be conceded specific powers by which the course of its appropriate nerve fibers would be so directed as to lead exclusively into that type of tissue and into no other. The mere fact that motor and sensory fibers, although coming from different sources, join each other and, as mixed nerves, follow common routes, speaks against the assumption of a separate and selective routing of fibers according to destination. Besides, the whole idea of specific "attractions" cannot very well be upheld in view of all the opposing evidence outlined above (p. 524). There seems to be little doubt but that the fibers while on their way have no par-

ticular aim and are unaware of where they shall eventually come to terminate.

2. The various tissues, though unable to attract nerve fibers from a distance, may still have their own choice in regard to the type of fibers with which they would enter into functional connection; from the mixed assortment of fibers indiscriminately invading the periphery each tissue would exclusively admit its appropriate set of fibers into intimate association, while all others would be rejected and pass by unconcernedly. This possibility cannot be definitely refuted at the present stage of our knowledge. Nevertheless, certain facts are known which do not seem to fit in with any such conception; foremost among them is the fact that nerve fibers do connect with tissues foreign to their kind if they are brought in close contact with them. Evidence for this has come from the field of nerve regeneration rather than nerve ontogeny, and this certainly weakens our argument. But since in other respects regeneration and first development are essentially comparable, the evidence may be admitted.

Counterevidence from heterogenous nerve regeneration.

It is a well established fact that severed nerve fibers can regenerate, and extensive studies have shown that in this process the two fragments of the fiber separated by the cut behave very differently.[1] The peripheral fragment, cut off from its central cell body, degenerates into plasmatic strands marking the old course of the nerve. The central fragment, on the other hand, moves to reproduce the lost peripheral portion: the protoplasm near the cut surface becomes mobilized, and soon another growing tip is established which begins to push out into the periphery, very much in the fashion of nerve growth during first development. While progress through most of the adult tissues is slow, degenerated peripheral nerve fragments furnish an excellent substrate for the regenerating branches to grow in, and at the same time offer smooth pathways. Since these deserted pathways are still attached to the same end-organs with which they were connected before transection caused them to degenerate, it becomes possible to force regenerating nerve fibers to grow toward any sort of tissue, simply by trapping them into the old tubes of degenerated nerves formerly connected with those tissues. The only question is, will the new fibers form connection with any sort of tissues, including those that are not of the proper type? To decide this, the following experiment was performed.

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[1] For comprehensive reviews of the problems of nerve regeneration, see *Boeke*, 1935; *Huber*, 1922.

Example 119.[1]

> A purely sensory and a purely motor nerve were severed and cross-united. Thereby the regenerating sensory fibers were forced into an old motor track, and the regenerating motor fibers were forced into a formerly sensory path so that each fiber type when arriving at the end of its new pathway was confronted with periphery belonging to the other. Motor fibers came to lie opposite sense organs, and sensory fibers arrived in muscles — both quite inappropriate destinations. Inappropriateness notwithstanding, the fibers proceeded to establish *terminal connections with the wrong kind of tissue.* It is significant to note that the structure of the new nerve endings conformed with the character of the peripheral tissue rather than with that of the nerve fiber; the termination of a *sensory* nerve fiber on a *muscle* fiber, for instance, resembled in all major aspects an ordinary *motor* nerve ending.

These experiments show quite conclusively that nerve fibers and peripheral tissue can unite rather promiscuously; no particular selectivity seems to control the admission of fibers into the tissues.[2] Inasmuch as the results pertain to regenerating rather than to developing nerves, they cannot, of course, definitely disprove that there might not be greater selectivity in the embryo, but they certainly discourage this view. So, the fact that at the end of development fibers and tissues appear correctly matched is still in want of an explanation.

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[1] *Boeke,* 1916, 1917.

[2] See also *P. Weiss,* 1934b.

Corrective influence of function.

3. Might it not be that nerve fibers and peripheral organs become first connected indiscriminately, whereas later, under the correcting influence of function, order would be brought into the system by the gradual elimination through degeneration of all incorrect and nonfunctional connections? Again the answer is negative; although some observations point to the possibility that there may be during embryogeny an initial overproduction of fibers followed by reduction, nothing has indicated yet that function plays any part in the process. On the contrary, it is well known that functionally ineffective junctions between nerve fibers and tissues may persist indefinitely.

Importance of time factor.

4. There is finally a possibility that the problem may be solved by taking into account the fact that different types of nerve fibers develop at different times. It is a fact that the first motor fibers grow out and reach the periphery long before sensory fibers develop. With this in mind, one could then reason as follows: Suppose all pathways available at the time when pioneering motor fibers start out on their journey lead into the musculature; suppose further, that the sensory fibers, which arrive at the periphery after a considerable lag, go past the occupied motor fields, because those pathways which are available at that time lead into the skin; and suppose, finally, that all later sensory and motor nerve fibers follow the pathfinders of their own kind because of some sort of specific "thigmotaxis"—then, a selective relation between motor fibers and muscles on the one hand, and between sensory fibers and sensory end-organs on the other, would result. As in so many other embryonic events, the success would be conditional on correct timing.

This last conception is rather schematic; its application to the concrete situation encounters a number of serious difficulties, and except for the time difference between motor and sensory fibers, which is an established fact, it rests upon pure speculation. A more penetrating experimental investigation of the problem is badly needed; it may disclose other possibilities hitherto unsuspected.

Determination of nerve endings by the innervated peripheral tissue.

There are marked morphological and functional differences between motor and sensory nerve endings and there are also striking differences between different kinds of sensory endings. Under normal conditions, when end organs and nerves are properly matched, it is impossible to decide which one determines the particular character of the junction: the tissue or the nerve. But since nerve fibers, as we have heard, can be united experimentally with tissues of other kinds than their own, the question could be solved. The character of the nerve endings was found to correspond always to the peripheral tissues on which they were formed, rather than to that of the nerve fibers forming them. It has been reported in example 119 that the ending of a sensory fiber on a muscle is of the motor type, and the ending of a motor fiber on a sensory tissue is of sensory character. Still more instructive is the following case.

Example 120.[1]

The skin of the duck's bill contains numerous sense organs of a unique type, the so-called corpuscles of Grandry, which are found nowhere else on the body. These sense organs, like many others, degenerate after the transection of their nerves (see p. 446), but can be re-formed in the course of nerve regeneration. Experiments were undertaken to test what would happen if the regenerating nerve fibers were to come from a strange source. A flap of skin was transplanted from the bill to the leg. The transplant, in which all Grandry corpuscles degenerated in consequence of the loss of innervation, soon became invaded by regenerating fibers from the local limb nerves which had been disrupted in the operation. These regenerating limb fibers caused the redifferentiation in the bill graft of Grandry corpuscles and properly connected with them although under natural conditions they would never have had any relation with sense organs of this kind. In other words, *limb* nerves in *bill* skin produce *bill* endings instead of *limb* endings.

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[1] *Dijkstra*, 1933.

It results from these experiments that the leading role in the determination of a nervous end-organ is carried by the peripheral non-nervous elements upon which the nerve fiber terminates. No matter where the fiber comes from, the peripheral tissue makes it turn out the appropriate kind of ending. Obviously this qualitative effect of the tissue on the nerve fiber could be classified as one of those processes commonly called "inductions" (see p. 335).

Specific functional correspondence between nerve centers and muscles.

The dominant influence of the peripheral tissue upon the nerve fiber is not confined, however, to the relatively modest role of determining the structure of the nerve ending. There is evidence that it is much more far-reaching, in fact, that it transforms the whole nerve. This must be concluded from the so-called "homologous response" of supernumerary muscles and limbs, which has shown that the various muscles of the body have each a specific individual characteristic which they impose upon their nerves. The situation is as follows. The central nervous system, when producing a coordinated movement, activates only a certain selection of muscles and each one with proper intensity. In order to be able to do this, it must obviously, first, "know" where the individual muscles are and, second, possess means of communicating with each one separately, just as the commandant of a military unit must know where his men are and must have ways of transmitting orders to those he needs in action.

The communication lines between the central nervous system and the muscles are the peripheral nerves. But how does the central nervous system "know" which nerve leads to what muscle? One may answer: it "knows" it owing to the inherited stereotypism of the connections, the hook-up supposedly being identical in all individuals, the same nerves always leading to the same muscles. But this supposition is erroneous. There is a considerable range of variability among individuals in the connections between nerves and muscles, which, after all, cannot surprise us if we remember the unspecific methods employed in guiding the nerves to their destinations. But quite aside from this objection, experimental evidence has demonstrated that the power of the nervous system to identify and control each individual muscle correctly does in no way presuppose a fixed and prearranged distribution of nerves.

Example 121.[1]

Transplanted muscles as indicators of selective relations.

In developed toads, a limb muscle was severed from its nerve, cut out, and transplanted to the back; there it was connected with a strange nerve that had formerly innervated some other limb muscle. The inserted nerve fibers effected new motor connections with the transplanted muscle and thus brought it again under the control of the central nervous system. After reinnervation, the transplants took part in the activities of the host animal; they contracted at certain definite phases, and when their function was studied more accurately, a remarkable fact came to light: Each transplanted muscle acted as if it were still in its old place and had still its old nerve. If, for instance, the transplanted muscle was a gastrocnemius muscle, experimentally connected with the nerve of an antagonist, it contracted always together with the original gastrocnemius muscle present in the normal limb of the same side. If, in another case, the transplant was a semitendinosus muscle, it contracted in unison with the normal semitendinosus muscle, and so forth. In other words, we find that *a supernumerary muscle is always*

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[1] Review and literature in *P. Weiss*, 1936.

made to act by the centers together with the normal muscle of the same name. This has been called "homologous response." If an entire supernumerary limb has been transplanted, the rule of homologous response makes each one of its muscles function together with precisely the *synonymous* muscle of the near-by normal limb, and consequently both limbs exhibit corresponding movements (Fig. 122).

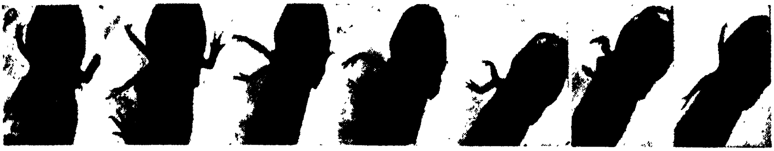

FIG. 122. Homologous response of synonymous muscles. Seven phases of a single movement of a salamander with grafted extra limb at left shoulder. The graft (T) is a right arm, hence a mirror image of the near-by original arm (O). The perfect synchronism and symmetry between the movements of O and T indicate that at any instant the same muscles are active in both.

The muscle "specifies" its nerve.

Since the central nervous system thus proves capable of dealing with each muscle as if it knew its *name*, even though the muscle had been transferred to a strange position and a strange nerve, it is obvious that there must be some way for the centers to establish the identity of the various muscles *after* they have become connected with their nerves. That the necessary information does not come by way of sensory messages over the sensory nerves has been demonstrated experimentally. The conclusion is inevitable that each muscle has a specific personal constitution, presumably of biochemical nature, distinguishing it crucially from that of other muscles, and that, by virtue of this property, it modifies its nerve fibers in such a specific fashion that they will become recognizable to the centers as belonging to that particular muscle. A fiber connecting with a gastrocnemius muscle acquires gradually a specific gastrocnemius tint, as it were, and, similarly, all other fibers acquire the stigmata of their peripheral muscles. These specifying effects encroaching from the periphery upon the fibers seem to extend proximally way down into the centers. This, then, constitutes the method by which the central nervous system gathers its detailed clues as to which muscle lies at the end of which line.

We realize from these facts that the establishment of nervous control over the periphery is a bilateral act performed in two steps: first, the central nervous system sends out the fibers into the periphery, and second, the periphery sends some specific influences back over the fibers into the centers. Both parts are indispensable for orderly function.

THE QUANTITATIVE DEVELOPMENT OF THE NERVOUS SYSTEM

Differences in size and numbers of nervous elements.

Once the pathfinder fibers have reached the various tissues to be innervated and have effected terminal connections with their end-organs, we can regard the peripheral nerve pattern as laid down in its gross lines. All further development involves mere elaboration of this primary pattern. Up into late embryonic stages there is incessant production of new neuroblasts which, in turn, give rise to new fibers which partly swell the intracentral fiber tracts, partly are poured out into the periphery. Inasmuch as all these later products simply follow the roads of their predecessors, the geometry of the nerve pattern has by that time ceased to be a problem. Instead, *numerical* problems have arisen. At what rate does the production of new fibers continue, and in what quotas are the newcomers added to the various existing nerves? Are they distributed at random or uniformly over the whole periphery? Or do different parts receive different allotments in proportion to their size or importance or functional versatility? And if there is regularity of numbers and distribution, then, what are the controlling factors? That there is such regularity, there can be no doubt; regarding its control some light has come from defect and transplantation experiments in which the normal relations of centers and periphery were altered; regarding the nature and operation of the controlling factors, nothing has yet become known.

Normal correlation between size of periphery and size of centers.

The most suitable object for a quantitative study of nerve development is not the brain but the spinal system with its segmental arrangement;[1] the segments represent a series of essentially equivalent units which can be compared directly with each other. Since the initial stage of all segmental nerves is approximately the same,[2] discrepancies discovered at later stages can be ascribed to segmental differences in the rate of new fiber outgrowth.

When we study an adult animal, we become aware of quite conspicuous and typical size differences between various levels of the spinal cord. The cord bulges at the levels of the fore limbs and hind limbs, becomes attenuated in the intermediate trunk portion, and finally tapers into the tail. The spinal ganglia show a corresponding variation in size. It is evident that these central differences reflect roughly the inequalities in the mass of peripheral innervated tissues; the limb segments which innervate a large mass of muscles and skin are much more strongly developed than the trunk or tail segments with their relatively smaller periphery. Considering final size as an expression of the intensity of past growth (see p. 421), a certain correlation between the growth of a peripheral organ and that of its innervating centers is thus clearly indicated. Whether this correlation is merely another case of developmental coincidence or is the product of a direct causal connection, can be decided from the following experiments.

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[1] Much of our present knowledge of the quantitative development of the centers has come from the concerted experimental work of *Detwiler* and his collaborators. This work is concisely reported in *Detwiler's* book on Neuroembryology.

[2] To be exact, one must take into consideration that the segmentation of the body proceeds in antero-posterior direction. Hence, more anterior segments are older and further advanced in their differentiation than are more posterior ones.

Example 122.[1]

Nerve centers after experimental reduction of peripheral tissue.

> In embryos of urodele amphibians (Amblystoma) the development of one fore limb was suppressed by extirpating the limb bud and covering the wound with skin in order to prevent regeneration. The fore limb of the opposite side was allowed to develop as control. When the two sides were later compared, the segmental nerves of the limb region were found to be considerably smaller on the operated side than on the control side. When the motor and sensory elements were counted separately, it was noted that the whole deficit was on the sensory side. The *spinal ganglia* of the limb segments contained considerably fewer cells on the operated side than on the control side, whereas no asymmetry was found between the left and the right halves of the *spinal cord.* In other words, motor fibers had grown out in equal numbers on both sides, while *the number of sensory fibers was reduced on the limbless side* (cf. Fig. 117).

There can be no doubt, therefore, that, at least with regard to the sensory innervation, the magnitude of the central development is linked with the actual mass of peripheral tissues to be innervated.

The suspicion that the condition of the periphery may have a direct bearing on the quantitative development of the nervous system was originally raised by observations on human individuals born with deficient limbs and showing corresponding underdevelopment of the motor limb region of the spinal cord (example 6, Fig. 20). In contrast to these human cases, however, the experimental cases just cited showed the effect confined to the sensory parts with no involvement of the motor parts. This discrepancy has turned out to be due to the fact that different species behave quite differently in this regard. When the experiments were repeated on the *chick* instead of the salamander, there was, besides a sensory deficit, also a marked reduction in the number of motor elements on the limbless side (Fig. 123),[1] just as in the human cases. We learn, therefore, that in higher vertebrates the correlation between actual size of the periphery and size of the corresponding parts of the nervous system extends to both sensory and motor elements, whereas in lower vertebrates it seems confined to the sensory parts.

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[1] *Detwiler*, **1924.**

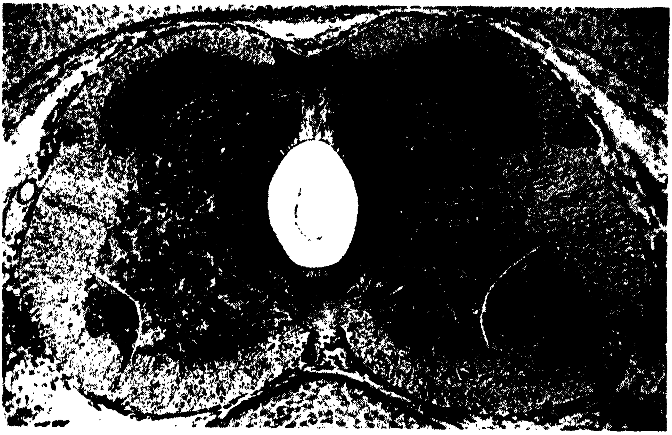

FIG. 123. Hypoplasia (reduction in cell number) of motor nerve centers following peripheral defects. (From *Hamburger*, 1934) Cross section through the limb level of the spinal cord of a chick embryo of 8 days' incubation which had suffered the extirpation of one wing bud (in the picture, left) 5 days earlier. The left half of the cord is smaller than the right half, and there is a striking deficit in the number of left motor horn cells (total for the wing region: left, 2044 cells; right, 5231 cells). The approximate limits of the motor horns are indicated by white lines.

Nerve centers after experimental augmentation of peripheral tissue.

The observation that growth of the limb centers remains subnormal when the development of the limbs has been suppressed suggests that the excessive development of the limb centers in normal animals is caused directly by the growth of the peripheral limbs. It should be possible to bring out the positive side of this growth-stimulating effect by allowing limbs to become connected with centers whose normal growth rate is relatively low. These experiments have, in fact, been performed and have led to the expected result.

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[1] *Hamburger*, 1934. Similar results have been reported for anuran amphibians (*May*, 1933).

Example 123.[1]

> If an embryonic limb bud is transplanted to the flank of the body some distance behind the original limb site (see also example 115), it receives at least a major part of its nerve supply from trunk segments, that is, centers which normally would innervate only a moderate amount of skin and musculature. Meeting the demands of this sudden increase of peripheral load, the trunk ganglia have reacted with an *appreciable increase of their own size.*[2]

According to these results, there can be no doubt about the fact that the development of the periphery has a definite repercussion upon the quantitative development of the nerve centers; of the nature of the effect and the way in which it is transmitted we are still ignorant.

Intrinsic size differences among different spinal regions.

It would be incorrect, however, to ascribe the enlargement of normal limb segments wholly to a direct stimulating effect of the limbs. The limb segments of a limbless specimen, while undersized, are still decidedly larger than any of the trunk segments. So the central limb region must after all possess an *intrinsic* tendency to relatively more intensive growth. It is on top of this basically higher growth rate that developing limbs, if present, exert their stimulating effects. Summing up, the quantitative development of the spinal centers appears to be a composite affair, controlled partly by intrinsic, partly by peripheral factors. The origin of the peripheral factors has been discussed; let us then turn to an examination of the intrinsic ones.

Intrinsic growth differences among different levels of the

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[1] *Detwiler,* 1920.

[2] Many processes may contribute to the increase in size: accelerated cell proliferation, intensified recruiting of indifferent cells for differentiation into neuroblasts, increase in cell size.

spinal cord date back to very early growth patterns such as the ones we have mentioned above for the brain (p. 505). However, with the progress of development, more and more secondary interactions among various parts of the central nervous system enter the scene and modify the original pattern. These interactions have been brought to light by experiments in which different sections of the neural tube were mutually exchanged or otherwise displaced. If the intrinsic growth rate of a given level of the neural tube were a matter of pure self-differentiation acquired in very early stages, one should expect that a spinal segment would invariably attain its normal dimensions, save for the damage due to the operation, and always the same dimensions regardless of its position and relation to the other segments. But transplantation experiments have failed to bear out this expectation. The experiments in question were done on urodele amphibians in which, as one remembers, the growth of the spinal cord is wholly independent of size differences of the periphery.

Example 124.[1]

Size adjustments in grafted spinal cord fragments.

A cylindrical piece of neural tube, several segments in length, was excised and replaced by a similar fragment of equal length taken from another embryo and chosen from a level the prospective growth rate of which was known to differ markedly from that of the replaced piece. For instance, limb segments in which growth is normally abundant, were substituted for trunk segments of comparatively low growth rate, and vice versa. The transplanted inserts developed in continuity with the host spinal cords into which they had been incorporated. Later measurements revealed that they had grown to *sizes characteristic of their new rather than their original locations.* Limb segments intercalated between trunk segments remained considerably smaller than normal limb segments, and trunk segments

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[1] Reviewed in *Detwiler*, 1936.

substituted for the limb segments of the cord reached much greater dimensions than are usually attained by trunk segments.

This result proves two things: first, that by the time the transplantation was performed, the transplanted sections had not yet been endowed with a growth tendency of definite magnitude; and second, that their growth rate has afterwards become adjusted so as to correspond to that typical of the particular region into which they had been shifted. In order to localize more closely the seat of the factors involved in the adjustment, the experiments were extended to include parts of the hind brain.

Example 125.[1]

Lack of size adjustment in brain grafts.

A number of segments were excised from the anterior end of the spinal cord directly behind the prospective hind brain. Into the gap a second prospective hind brain region taken from another embryo was inserted, so that a tandem arrangement of two prospective hind brains was obtained. In its original setting the transplanted piece would have given rise to a medulla oblongata. There is a marked difference of shape and size between a medulla oblongata and any portion of the spinal cord. As for size, the medullary levels grow to larger dimensions than even the limb levels of the cord. Accordingly, if the results of example 124 were of equal validity for all parts of the central nervous system, a hind brain substituted for spinal segments should remain inferior in development to the host's own hind brain. Quite to the contrary, however, *the grafted prospective medulla turned out to be approximately as well developed as the normal medulla lying in front of it.*

There is, therefore, a significant discrepancy between the results obtained by transplanting spinal segments and hind brain fragments; in contrast to the former, the latter exhibit a remarkable degree of quantitative independence and produce a fragment of medulla much the same as if they had

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[1] *Detwiler*, 1936.

been left in their original location. Their growth rate, unlike that of the spinal cord, must have been fixed prior to the stage of transplantation. There is another striking difference between fragments of spinal cord and medulla, when grafted to the same strange levels; while the former submit passively to growth-controlling influences of their surroundings, the latter spread an active growth-promoting effect upon the normal spinal segments with which they have become linked. As a result of this the spinal segments lying behind the transplanted extra medulla develop to sizes in excess of normal, without having been moved from their normal positions.

Intracentral interactions.

On the whole, the effects observed in these two sets of experiments (examples 124 and 125) are complementary. They reveal that there are significant differences in the state of activity and reactivity among different portions of the embryonic nervous system. As is generally true in development, plasticity goes hand in hand with lack of self-differentiating tendencies (example 124), while self-differentiating capacity, conversely, implies some power to control less advanced parts (example 125). It must be understood that "self-differentiation" pertains in the present example to one character only, namely, the growth rate. Save for this limitation, the resemblance between these results and those obtained with grafted fragments of the early germ is striking. One is reminded at once of the chorda-mesodermal field district of the blastula with its precocious capacity for self-differentiation attended by the faculty to exert "organizing" influences upon more plastic regions. This parallelism in principle does not necessarily imply identity of the underlying mechanisms.

We do not know the exact mechanism by which certain nerve centers (e.g., medulla oblongata) affect the growth rate of others (e.g., spinal segments). But tentatively the following idea has been advanced. It has been suggested that intracentral nerve fibers growing down the length of the cord may provoke an excess proliferation of nerve cells at the level at which they come to terminate.[1] According to this view, the growth stimulation of any spinal region would be proportional to the amount of nerve fibers ending in it. Since the bulk of the intracentral fiber tracts arises from supraspinal, i.e., brain, regions, with the medulla figuring as a major source, a transplanted fragment of medulla would necessarily stimulate the growth of the adjoining spinal segments into which it is allowed to pour its fiber masses (example 125). In order to account, however, for the very definite distribution of growth rates along the axis of the spinal cord (for instance, the intrinsic increase in the limb segments and decrease in trunk segments), one would have to postulate that each descending fiber has its prescribed measured length at the end of which it would cease to grow. Whether this really is the case has not been ascertained. The mere fact that a correlation between local growth rate and number of arriving nerve fibers exists, does not reveal whether cell proliferation is increased because more fibers terminate or whether, contrariwise, more fibers terminate because of an increased local rate of proliferation, increased for reasons other than fiber ingrowth. The decision hinges on the more general problem of what makes a fiber terminate, and this problem is, as we have indicated before, still unsolved. So, the possibility still remains that the agents controlling the growth pattern of the spinal cord in later stages may not, after all, be so essentially different from the factors determining embryonic growth patterns of earlier stages as one would have to postulate if fiber ingrowth were the dominating factor in the former.

The matter is still further complicated by the fact that transplanted spinal fragments assume not only the quantitative aspects of the sections for which they have been substituted but also their functional properties.[1] Trunk seg-

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[1] *Detwiler*, 1936.

ments grafted into the place of limb segments (example 124) differentiate the complete functional apparatus required for the central control and coördination of limb movements. Mere fiber ingrowth could certainly not engineer a product so specific as this. In view of the many unsettled points, it seems impossible to take any definite stand; the indicated thing to do, at present, is to carry the factual analysis further, and, pending its outcome, keep an attitude of reserve.

CONCLUSIONS

Our discussion can have left no doubt but that our information about even the elementary facts of nerve development is still in a very fragmentary state. Wide gaps of unexplored territory separate the few spots upon which critical observations and analytical experiments have shed some light; even this light is often enough an ambiguous twilight which makes the facts assume quite different aspects in the eyes of different authors. In this state of affairs, an attempt to reconstruct the whole story from the few available fragments cannot be expected to produce results incontrovertible, final, and worthy of unrestricted credence. Nor has it been our purpose to draw up a story of ultimate validity. What we intended to show was simply that there is a way for rational and more than verbal approach to the problems of nerve development, and that it will pay to continue to work along these lines.

The composite character of neurogenesis.

At least one point, however, has already been established safely and definitively, and that is the highly composite character of neurogenesis and the multiplicity of factors engaged in it. The thesis heading this chapter has been fully substantiated by the facts; the "development of the nervous system" is not the simple, continuous, unitary event as which it presents itself to the non-analytical observer. We have demonstrated how it has been decomposed through analytical investigation into numerous heterogeneous and independent constituents. The construction of the nervous system does not lie within the province of a single craft; it resembles the fabrication of a complex product in a modern industrial plant in which many and diverse kinds of activities take part.

The coördination of events in space and time

Naturally, where there are many hands coöperating toward a common goal, the harmony of the production is only insured provided the individual activities are strictly coordinated according to a definite plan. Each partial process must come in at the proper time and in the proper place. In industry the plan is premeditated; in the organism we assume it to be a product of evolution. The presence of a definite space and time pattern as a sort of frame into which the numerous component activities are fitted is prerequisite for organization; its absence precludes organization altogether. A change in the spatial relations or in the timing of just one contributory process can disrupt the whole sequence.

THE DEVELOPMENT OF BEHAVIOR

Nervous development is antecedent to nervous function.

The nervous system reminds us of an industrial plant not only by the multiplicity of cooperating agents and the harmonious coordination of their activities, but also by the fact that the construction is completed in its main lines without the experience, and, in fact, even before the onset, of actual function. A ship at the time of its launching represents best the state of the nervous system at the time when it first engages in functional activities; though much remains to be perfected, it is able to float, move and steer. Similarly, the nervous system, when for the first time called upon to act, can already conduct and coordinate impulses and control the musculature, at least grossly. In this respect studies of the nervous system have simply corroborated *the pre-functional character of development in general.*

However, this truth has not always been recognized, either in behalf of the organism as such or, and much less so, as applying to the nervous system. It has often been contended that the nervous system of the individual starts its functional career from a rather homogeneous undifferentiated condition upon which individual experience, i.e., the molding effect of actual functioning, would gradually inscribe more definite patterns; through trial and error, by the ingraining of patterns that turn out to be successful, and the erasing of those that have proved to be failures, the whole organization of the nervous system would be built up by degrees.

This claim was raised mainly on the strength of psychological and comparative-anatomical arguments. Characteristic traits observed in the ontogenetic as well as phylogenetic development of mental functions were projected upon the nervous system legitimately considered as the substratum of those functions. Mental associations were assumed to involve the formation of material cell connections in the brain, and the notable increase in wealth of associations and discriminatory abilities in a growing mind, which is a commonplace fact, was interpreted as being the counterpart of an increase in the number, complication and differentiation of central pathways, shaped by actual functional activity. This view, gained from an interpretation of the highest functions of the brain, was then extended to accommodate the lower functions of the nervous system as well, down to the simplest reflexes.

The concept of a post-functional, acquired, character of nervous organization has been steadily controverted, however, by a group asserting its pre-functional, innate, character. According to their view, all those properties of the nervous system which enable it to perform its regular functions are developed prior to, and in the absence of, all function. If function was to be conceded any part in development at all, it was in the elaboration and further perfecting of existing patterns rather than in the creation of new ones. For a long time the controversy remained on rather philosophical grounds, hence unsettled. Nowadays, a sufficient number of concrete facts have been collected to permit a decision; and the decision has been in favor of the *pre-functional* thesis.

Alternative methods in the study of the ontogeny of behavior.

The problem of the development of behavior can be approached by two roads: One way is to study the onset and subsequent changes of behavior as it manifests itself to immediate observation, irrespective of its underlying physiological mechanisms. The other way is to follow the development of those anatomical structures and physiological properties which are known to be, or suspected of being, related with behavior; i.e., principally the nervous system and the organs under its control.

Observational studies of behavioral development.

Both ways have their justification and merits. The former has the advantage of being more direct. Comparing, for instance, the movements of babies at various stages, one records a progressive development from the sweeping, ill-aimed, ill-controlled use of the members during earlier phases, toward smoother, localized, better coordinated application at a later time. The gradual establishment and improvement of elementary reactions, such as eyelid closure, convergence of the eyes, grasping, and the like, may be followed even quantitatively, and in the end of such comprehensive studies, one arrives at certain generalizations: laws of the post-natal development of human behavior. Extension of these studies to animals broadens their scope and usefulness because the examinations can be carried back into pre-natal stages. The behavior and reactions of the mammalian fetus can be observed after dissecting the exposed uterus. Embryos of egg-laying forms are, of course, still more readily accessible to direct study.

This commendable field of research, although still in its early beginnings, has already uncovered some fundamental facts concerning the ontogeny of behavior. In the first place, it has confirmed the contention that the behavior displayed by an animal at the time of its hatching or birth, as the case may be, has not arisen in a sudden single step, but is the product of a long developmental history, just as are the other morphological and physiological features of the body. When an animal emerges from the enclosure of the egg, or of the maternal organism, to carry on free life on its own, we find it already in possession of fairly advanced behavioral capacities. It perceives and discriminates certain stimuli of the external world and reacts to them in relatively adequate fashion, and it is fit, within certain limits, to meet the exigencies of its new environmênt, if they are not too exacting. These are functional activities of some degree of diversity and complexity. What is their origin? That the unborn child stirs in the mother's womb, or that an unhatched chick moves in its shell, has, of course, long been known. But the character of these pre-natal movements and the mode of their appearance was not revealed until recently when they were traced back through the earlier embryonic stages to their very source. Then, it became evident that *the complex patterns manifested at birth are preceded by an orderly sequence of more primitive reactions* which are the simpler the further back one goes in the embryonic history.[1]

Development of behavior in amphibian embryos.

For convenience, the movements of an animal may be classified according to their incentives into spontaneous and reflex, or actions and reactions. The distinction is rather subjective, but, on the whole, reactions elicited by identifiable sources of stimuli can be designated as reflexes, while actions resulting from indiscernible factors located inside the animal may be termed spontaneous. Both have closely related embryonic histories, but since spontaneous activities are beyond our control, because they cannot be elicited at will, it seems more suitable to base a behavioral standardization of developmental stages on reflex reactions. The gradual development of reflexes in the Axolotl embryo may serve as a first illustration.

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[1] The first comprehensive treatise on the development of physiological functions in the embryo was published by *Preyer* in 1885 (*Specielle Physiologie des Embryos;* Leipzig). An English translation of the chapters on motility and sensitivity has been furnished by *Coghill and Legner* in: Monogr. Soc. f. Res. in Child Developm., vol. *2*, No. 6; 1937.

Example 126.[1]

The first muscular contractions of an axolotl embryo are observed at a stage when the general form of the body has barely been laid down and the differentiation of organs is still in a relatively primitive phase. Strong shocks, mechanical or electrical, elicit muscular twitches that can hardly be classed as movements; responses to touch applied to the skin are still absent at this stage. Such responses appear only later and consist, at first, of a slow, protracted flexure of the head, strictly localized to the neck region (1st motile stage). The failure of other parts of the body to react indicates that they are not yet ready for functional use. As development goes on, the movement becomes increasingly more extensive, until eventually the whole side of the body takes part in the flexure, rolling the embryo into a coil. The application of an external stimulus produces a wave of contraction which sweeps over the trunk musculature of the opposite side in antero-posterior direction (stage 2). Stage 2 marks a decided progress over stage 1 inasmuch as the excitation spreads over a greater length of the body, although it remains still confined to one side. The next development is that both sides become involved in the response. While the first wave of contraction travels caudad on the side opposite to the stimulated one, another wave appears with a lag on the stimulated side; the combined action of both waves throws the body into the shape of an "S" (stage 3). The final step in the establishment of primitive locomotion is accomplished when, slightly later, several "S" reactions follow each other without interruption, producing fish-like swimming movements (stage 4). Every movement now engages the whole body. The first gill responses which soon appear, are also associated with comprehensive body movements. Similarly, the limbs, when they become functional, move at first in unison with the trunk. Only after some more time has elapsed, can independent and isolated responses of the limbs, gills, and jaws be obtained; this *gradual functional emancipation of parts from the rest of the body* seems to occur in a definite sequence, *leading from more widespread to more localized reactions.*

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[1] *Coghill*, 1929.

The gradual emergence of behavioral patterns.

The story told by these observations consists plainly of two parts. The first concerns the earliest functional stages; it relates the very primitive, local, stereotyped character of the incipient reactions; the gradual increase in their comprehensiveness, complexity, and duration; and the resulting stage of a body engaged "with every fiber" in the acts of locomotion. Then comes the second part: the gradual restriction and restraint in the use of the sweeping capacities previously gained; instead of all parts being involved in all actions, only those immediately concerned are activated, which procures greater variety, finer differentiation and better economy of movement. There are, thus, two periods in embryonic history during which responses are localized rather than sweeping: one at the very beginning of function when the functional apparatus is still fragmentary, and another one at the end of functional development when local functions have acquired dissociability from the functional background of the remaining body. While the former phase is expansive, expressing the steadily increasing proportion of serviceable parts, the latter phase is restrictive in that during it barriers are set up through which the various organs become functionally detached from each other. Henceforth each organ can be activated alone without engaging the others.

It will be noted that this secondary dynamic emancipation of parts from primary integral bonds and the breaking up of more comprehensive functions (total body reactions) into a number of more narrowly restricted functions (individual organ reactions) reflects precisely the general character of embryonic development as it has been reported above in Part Three. Furthermore, this has long been recognized to be the trend of the development of behavior during the post-natal period. If we take, for instance, the act of post-natal motor learning, we know very well that it starts with rather comprehensive movements; these are then gradually narrowed down in scope and extent by our becoming increasingly adept at suppressing waste motions. Everyone who has learned to play an instrument is familiar with the initial difficulty encountered in keeping the other fingers from moving along with the one finger that is supposed to move singly.

The general resemblance between pre-natal and post-natal behavior has certainly done much to promote the idea that both follow essentially similar principles so that the fundamental information collected in one field might legitimately be extended to the other.[1] The tendency to such far-reaching generalizations undoubtedly exists, and this makes it doubly important to ascertain whether the course of pre-natal behavior observed in the axolotl is really typical of all other animals as well, including man. Certain studies on the rat [2] and cat fetus [3] seemed to corroborate, on the whole, the picture obtained from the amphibians. The following case may serve as an example.

Example 127.[4]

Behavioral development in higher forms.

The uteri of pregnant cats of various terms of gestation were exposed, and the fetuses were immersed into a bath of saline solution of body temperature leaving the umbilical circulation intact. A graded series of developmental stages from 20 days of gestation until birth were thus made available for physiological

[1] *Carmichael*, 1936.

[2] *Angulo y Gonzalez*, 1932.

[3] *Carmichael*, 1934.

[4] *Coronios*, 1933.

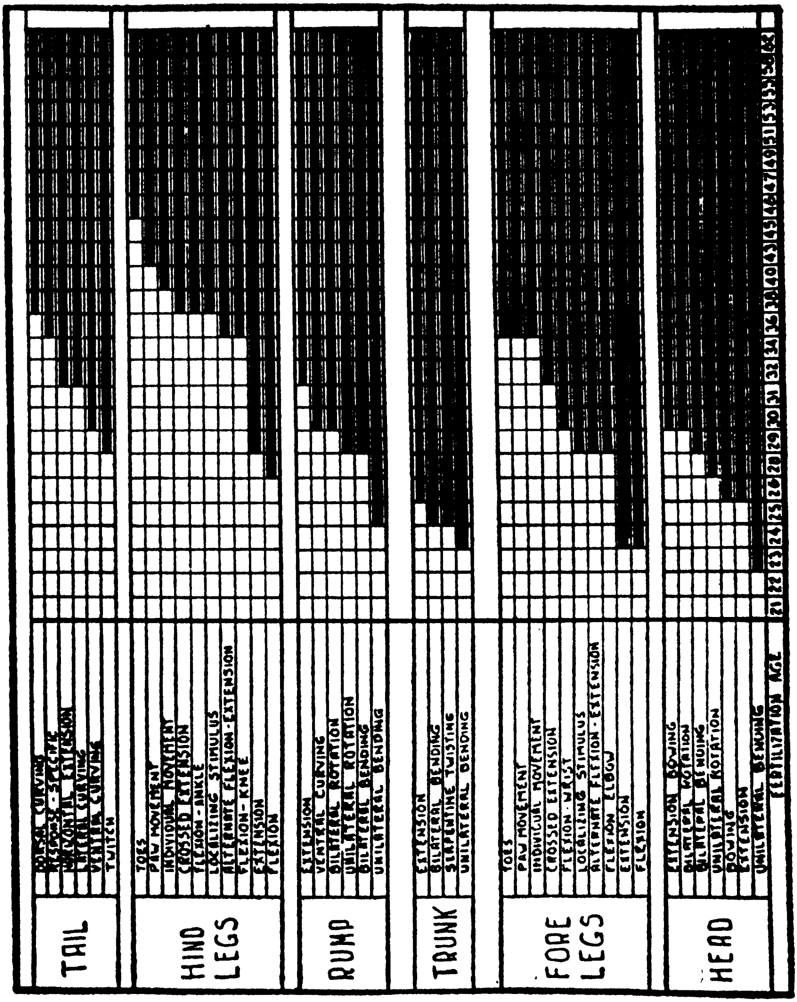

FIG. 124. Initiation of movements in the fetal cat. (From *Coronios*, 1933) The beginning of a bar marks the day (counted from fertilization) on which a given movement has first been observed.

tests. The specimens were mechanically stimulated in various places and their reactions were recorded. A digest of the results is reproduced in Figure 124. It brings out the following points: with increasing age of the fetus (proceeding to the right in the diagram) the wealth of reactions (black bars) grows steadily. This increase follows clearly a cephalo-caudal course; the more posterior levels lag behind the more anterior ones (compare especially head — forelegs — hind legs). Within each level, however, we notice a gradual differentiation of function which advances clearly in the direction *from crude and generalized movements toward finer and more circumscribed reactions.* Concurrently the various parts acquire independence of motion from the mass reactions of the body with which they were previously tied up.

Although there are differences in detail, this description can be fitted into the general conception suggested by the amphibian observations. Other authors, however, have come to contradictory conclusions. They have asserted that at least in some forms (cat,[1] chick [2]) the localized and well circumscribed reflex is and remains the primary behavioral reaction; they deny that local reflexes are originally merged into and only secondarily recovered from behavioral mass responses engaging the whole body. The controversy has not yet been settled, but it seems that differences of either the methods employed or the forms studied can account for the discrepancies.[3]

Does "learning" affect the development of embryonic behavior?

Whatever discordances there may exist concerning the details, there is certainly consensus about the general fact that behavior has an embryonic as well as a post-embryonic history leading through a transition of stages of increasing perfection from rudimentary beginnings to the fully functional condition of the adult. Since movements can actually be executed to the extent to which embryos or fetuses are mechanically free to move, the question arises as to whether this pre-natal functioning may not be tantamount to practicing. Do these movements provide the embryo with an opportunity to get acquainted with its motor apparatus and to "learn" to perform functional patterns of increasingly greater complexity under the guidance of its own experience? Or are the movements of the embryo merely aimless and pointless exhibitions of motor capacities developed by intrinsic law regardless of experience? In the latter case, the embryonic movements would be simply indicative of, but by no means necessary for, the progressive autonomous elaboration of the behavioral mechanism; in fact, they would be as irrelevant for the establishment of the basic functional patterns as the actual bite is for the production of poison in a snake's fang.

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[1] *Windle*, 1934.

[2] *Windle* and *Orr*, 1934.

[3] *Hooker*, 1936.

The clear-cut alternative points a way to decide the issue: one has to find out how an individual in which pre-natal motility has been suppressed will behave at birth. If embryonic movements are really indispensable as stepping stones for behavorial progress, such animals should be expected to turn out at birth with no motor repertoire. If, on the other hand, the behavioral apparatus develops autonomously, function or no function, the animals born would not be inferior to normal controls that had been functioning all the time. To begin with we can cite some rather elementary observations decidedly in support of the latter view.

Example 128.

Motor behavior without previous experience.

The position of a butterfly inside the pupal case is such as to preclude movements of the appendages; there is simply no space to move in. Nevertheless, the animal, after breaking the case, frees itself immediately by *well-coordinated movements*, creeps out of its envelope, spreads its crumpled wings, and flies off, as soon as they have dried, very handsomely, *without ever having had a chance of learning and practicing* the various performances of which it suddenly reveals itself to be fully capable.

Example 129.

Some salamanders are viviparous[1]; the eggs, of which there are several dozens in a female, complete development in the distended oviducts in such crowded condition that it is utterly impossible for an individual to stir during intrauterine life, which may last for several months. A litter can be delivered artificially before the term by killing the mother, excising the tubes and discharging the larvae into water. When this is done, the larvae, which had been lying distorted and motionless, assume instantaneously the well-coördinated activities typical of free-living animals, such as swimming, walking, orienting, breathing, feeding, swallowing, righting, and so forth. Every one of these acts, when produced for the first time, appears *in good order, although the animal could have had no previous experience with its execution.*

Development of behavior in anesthetized embryos.

One might object that the unborn larvae, although mechanically prevented from moving, could, nevertheless, innervate and contract the muscles and might by this modest means have acquired some familiarity with the potential use of their locomotor apparatus. This assumption, however, has been invalidated by the following experiments.

Example 130.[2]

Embryos of Amblystoma are free to move inside the spacious egg membranes from the very first signs of motility (see above, example 126). On the other hand, means have been found to suppress the motility without jeopardizing development. This is, therefore, an excellent object on which to test our problem. Amblystoma embryos, when reared in an anesthetic drug (chloretone) of suitable concentration, are found to continue to develop along normal lines, although at a somewhat retarded rate; at the same time they are completely paralyzed and do not exhibit any embryonic motility. They can be kept in this condition for many days, up to a time at which control larvae of identical

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[1] E.g., the European species, *Salamandra maculosa.*

[2] *Carmichael,* 1926; *Matthews* and *Detwiler,* 1926; first reported by *Harrison,* 1904.

> age raised in ordinary water have gotten far beyond the hatching stage and have started free life with swimming and feeding. If, then, the experimental animals are returned to fresh water and allowed to recover from the narcotic sleep, a very remarkable thing happens. As soon as the paralysis subsides, *the animals take up at once all the performances shown by normal animals* of the same stage of differentiation; they swim and feed like the controls, skipping the various phases of more primitive reactions through which a normal animal passes in the course of its development.

Since in this case embryonic movements have been prevented not merely by mechanical immobilization as in examples 128 and 129 but by the suppression of all nervous activity, it is clear that the development of the elementary mechanisms of behavior is independent not only of *effective* nervous function, but of nervous function as such. Any experience of the embryo with managing its body can apparently be dispensed with without threatening the establishment of normal reaction patterns such as we find ready for use at the moment of hatching or birth. One must conclude, therefore, that the fragmentary movements observed during the embryonic history of motile embryos merely gauge the gradual progress in the differentiation of the underlying functional apparatus without materially contributing to it.

The innate character of behavior.

To summarize, the gradual change from simple to complex in the pre-natal reactions of an animal is merely an outward expression of an autonomous progressive differentiation of the bodily mechanisms required for function, the steps of the progress being determined by *intrinsic* developmental factors quite unrelated to actual functioning. In this connection it is well to remember the analogous situation presented by the "functional" structure of bone, as discussed above (p. 457). Just as the architecture of the bone is not produced by the very stresses of load and resistance for which it shows itself so well prepared when it is later put into use, so the "functional structure" of the behavioral system cannot be regarded as a result of molding effects exerted by functional use upon a plastic and pliant body. On the other hand, we remember that the bone does undergo a certain degree of adaptive perfecting which is functional in origin; but this does not occur until much later, during post-natal life. Similarly, we must concede to the behavioral system the capacity of being later, secondarily, elaborated into greater detail, higher efficiency, and finer adjustments under the guidance of its actual operation. In order to rate these secondary improvements correctly, one must keep in mind that they compare with the ground-work of organization as the interior decoration of a building compares with its construction. In embryology, our main concern is the construction.

The development of the somatic basis of behavior.

Since, for the stated reasons, the development of behavior resolves itself into the development of its anatomical and physiological correlates, it might seem a more adequate procedure to study primarily the development of the nervous apparatus, including receptor and effector organs, and to ignore its behavioral exhibitions. This is the other of the two approaches mentioned above as being accessible in the study of the ontogeny of behavior. It encounters, however, a fundamental and, at present, insuperable difficulty; namely, that we are not yet able to reconstruct behavior from the known structural and physiological properties of the nervous system, even in the adult body, to which anatomy and physiology have devoted so much work. How much less can we hope to make rapid headway in this task in the embryo where our information is still so fragmentary! Physiological investigations of the embryo are practically lacking, and this lack can never be compensated by purely anatomical and histological studies of the embryonic nervous system, however complete these may be. Merely looking at a nerve cell or nerve fiber can never tell us whether or not it functions, and much less how it functions. In this state of affairs it seems expedient to continue along both lines and to pursue studies of the development of the nervous system and of the development of behavior collaterally, though the data recorded in the two fields may have to remain, for some time to come, in juxtaposition rather than correlation.

Incomplete function in incomplete nervous systems.

Exception must be taken to those cases in which *absence* at a given stage of a certain reaction can be definitely linked with the *lack* of some particular character of the nervous system. From observations of this kind inferences concerning the functional value of the lacking structure can often be drawn with reasonable safety. A few facts may illustrate this.

In most forms the first reaction to tactile stimulation is a localized muscular contraction confined more or less to the stimulated area (see p. 64). If this effect is obtained at a stage at which histological preparations fail to reveal the presence of nervous connections with either muscles or skin, it is evident that the muscles must have responded to the stimulus directly. Here the correlated physiological and anatomical investigation has, therefore, resulted in the demonstration of a *pre-neural stage of motility.*

An anatomical examination of the nervous system of the axolotl through the typical behavioral stages described above on page 562 (stages 1–4) has demonstrated that each following stage is superior to the preceding stage by the possession of some new type of nerve connections in the spinal cord.[1] Stage 2 (coil), compared with later stages, lacks cross-connections between the motor tracts of the two sides; this lack has been blamed for the restriction of the coil reaction to one side of the body. Stage 1 differs from the later stages by the fact that the longitudinal fiber tracts, growing in antero-posterior direction, have not yet reached the more posterior levels of the cord; this accounts for the failure of the head flexure to spread caudad, as it does later in stage 2.

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[1] *Coghill*, 1929.

More generally speaking, where there is no nervous pathway, there is no communication, and so long as the numerous local nerve centers have not become interconnected serially and crosswise, there can be no integrative nervous control over the individual parts which they represent.

The appearance of coordinated function.

While the negative angle of the morphological-functional conjuncture in nervous-behavioral development is thus quite definite, little is gained on the positive side. While the absence of certain nervous connections explains why certain reactions do not and cannot occur, the presence of certain nerve connections only explains why certain reactions *can* occur, but not why they *do* occur in a particular manner varying with the kind, strength, localization, and distribution of the stimuli, as well as with the internal condition of the body. Ubiquitous and indiscriminate excitation of all nervous structures present at a given stage would make an animal of that stage capable of one single type of reaction only. This seems, indeed, to be the case during the very first stages of functioning, as may be concluded from the stereotypism of the early reactions. But it is certainly no longer true in slightly later stages, when the reactions have ceased to be uniform and have become increasingly diversified. Then, differentiated responses appear which, instead of engaging the total mass of excitable elements, only activate appropriately selected groups in varying combinations. Now, in the search for a comprehensive and wholly acceptable answer to the problem of how this selective restriction of excitations in the service of coordinated function occurs, embryology has, in our opinion, not yet attained any greater success than physiology. Those schematic interpretations which attempt to reduce the whole problem to terms of "reflex arcs" laid down during embryogenesis as chains of neurones do not seem to fit any but the most simplified notion of the nervous system.

The ontogeny of functions as promising field of research.

Looking back on our discussion of the development of nervous functions, one becomes sadly aware of the primordial state in which this whole field still rests. In comparison to the wealth of facts known in morphological embryology, the dearth of information concerning the functional side is disconcerting. This disproportion is not surprising. Morphological embryology has received its major stimulus from a powerful idea — Evolution (see p. 118); physiological embryology has not yet found an equally potent sponsor. Of course, once physiology will have become aware of the profit it can derive from the study of embryonic functions, the situation may change abruptly. There are many advantages which the study of embryonic functions offers over that of the adult body; embryonic functions are more primitive, hence more schematic, and show the crucial aspects in sharper relief. Moreover, by choosing the proper stages, it is possible to get an unobstructed view of certain vital phenomena while they are still in pure and, as it were, nuclear form, not yet obscured by a host of complicating factors of more recent appearance. To get rid of the latter the physiologist must apply elaborate surgical and pharmacological measures while the embryo might have presented him with the unobscured situation from the beginning. Apart from the work on the nervous system, advantage has been taken of this fact only in the study of heart function and a few other scattered instances. A systematic exploitation of this promising field of Embryonic Physiology has not yet even begun.

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